Bioaerosol vertical fungal spores profile in Minas Gerais State, Brazil

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.

HAL Id: hal-03563153
https://hal.inrae.fr/hal-03563153v1

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

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

Affiliation:

[a]Institute of Astronomy, Geophysics and Atmospheric Science (IAG), University of São Paulo (USP), Brazil

[b]National Research Council of Italy (CNR), Institute of BioEconomy (IBE), Italy

[c]Adolfo Lutz Institute, Department of Micology, Brazil

[d]Laboratory of Research in Marine Environmental Monitoring (LAPMAR), Geosciences Institute, Federal University of Pará, Belém, Brazil

[e]Interunities Graduate Program in Biotechnology, University of São Paulo, Brazil

[f]Unité de Pathologie Végétale UR407, INRA, Montfavet, France

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

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

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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 (*Penicillum/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

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1. Introduction

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

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

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

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

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

On the other hand, hailstorms are one of the most crop destructive meteorological 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   <u>Sampling site</u>

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 (21º23'36.37" S and 46º55'16.48" W) and the other was in the northwest

129   of the Arceburgo city, were only the sample from day 30.07.19 was collected (21º18'48.25" S

130   and 46º50'40.44'' W). The ground samplings were collected immediately before the balloon

131   flight for bioaerosol measurements.
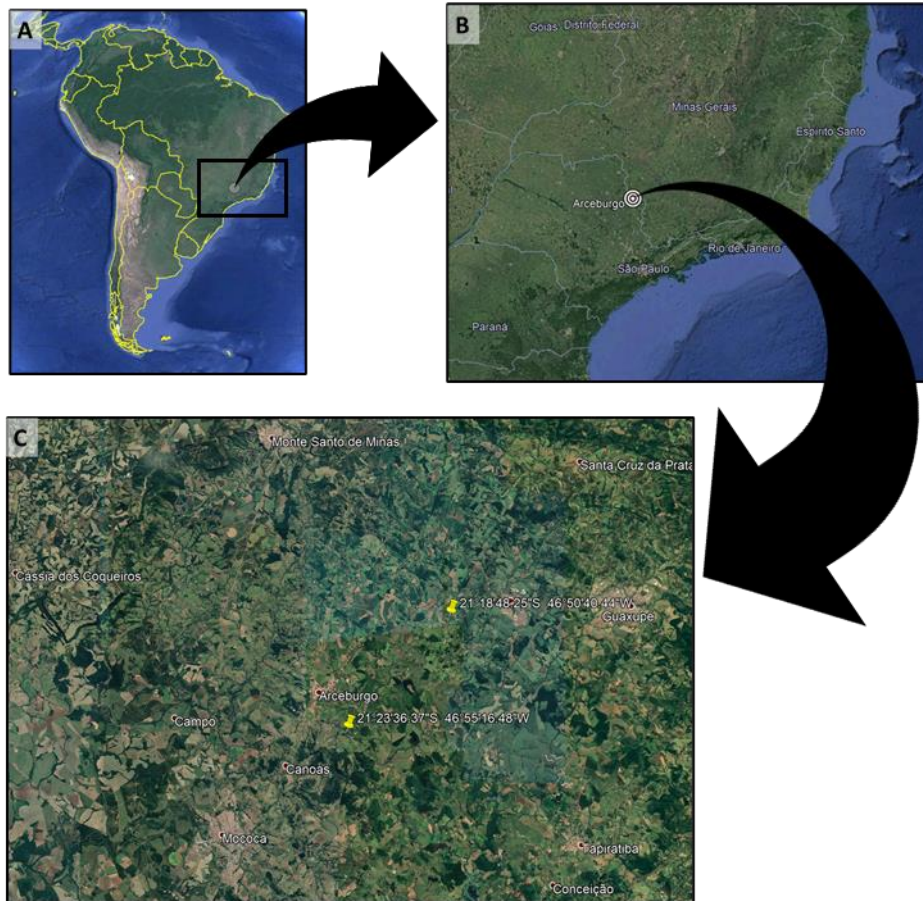
132
133 **Fig. 1** Map with the sampling sites. A: South America; B: Southeast of Brazil; C: Region of the
134 sampling site centered at the 21°23'36.37" S and 46°55' 16.48" W, and at 21°18'48.25" S and
135 46°50'40.44" W (yellow markers), nearby Arceburgo City (21° 20' 35" S and 46°56'21.56" W,
136 ~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 30ºC (± 2ºC) 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°x0.25°. 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 (±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 (ρ=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), *Aspergilium* 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 (±8,124) spores/m³ and 3,593 (±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).

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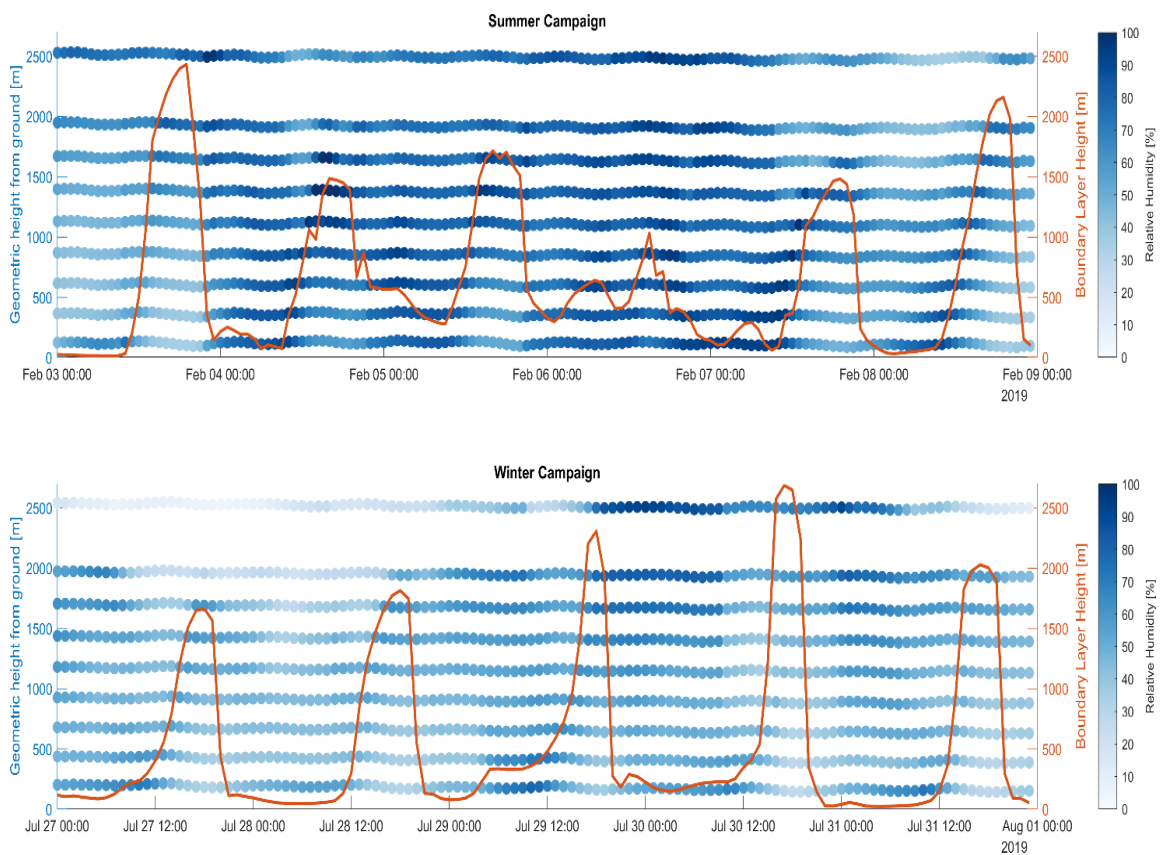
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284 **Fig 2** Relative humidity and boundary layer (BL) heights for the two sampling Campaigns.
285 Position of the colored dots indicates height above ground (left y-axis), while the intensity of the
286 blue color of the dots indicates RH percentage (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

290 ### 3.3. Overall Fungal concentration in different altitudes

291   Samples were collected in different altitudes in both campaigns (winter and summer),

292 with MAS100 and portable Burkard, however, only winter campaign concentrations were

293 considered in the comparison between altitudes. Results for the differences of fungal type

294 concentrations for each altitude may be found on table 1.

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
Alternaria **sp.**	+*	-*	+	-	+*	-*	+	-
Pen/Asp.-**like**	+	-	+	-	+	-	-	+
Cercospora **sp.**	0	+	0	+	0	+	+	-
Cladosporium **sp.**	+	-	-	+	+*	-*	+	-
Curvularia **sp.**	+*	-*	+	-	+*	-*	+	-
Drechslera-**like**	+*	-*	+	-	+*	-*	+	-
Helminthosporium sp.	0	+	0	0	0	+	+	0
Periconia sp.	+	-	0	+	+	-	+	-
Epicoccum **sp.**	+*	-*	+	-	+*	-*	+	-
Botrytis **sp.**	0	0	0	0	0	0	0	0
Nigrospora **sp.**	+	-	0	+	+	-	+*	-*
Pithomyces **sp.**	+	-	0	0	+	-	0	+
Spegazzinia **sp.**	+	-	0	0	+	-	+	-
Stemphylium **sp.**	0	+	0	0	0	+	+	0
Tetraploa **sp.**	+*	-*	0	+	+*	-*	+	-
Torula **sp.**	+	-	0	+	+*	-*	+	-
Unknown MS	+*	-*	+	-	+*	-*	+	-
Smuts	+	-	0	+	+	-	+*	-*
Coprinus-**like**	+*	-*	+	-	+*	-*	+*	-*
Agrocybe/Conocybe-**like**	0	0	0	0	0	0	0	0
Calvatia lycoperdon	0	0	0	0	0	0	0	0
Boletaceae	0	0	0	0	0	0	0	0
Ganoderma **sp.**	+	-	0	+	=	=	+	-
Hyaline Basidiospores	+*	-*	+	-	+*	-*	+	-
Panoellus/Psathyrella	0	+	0	+	0	+	+	0
Colored Basidiospores	-	+	0	+	-	+	+	-
Unknown Basidiospore	0	+	0	0	0	+	+	0
Puffball	0	+	0	+	0	+	-	+
Saccobolus	0	+	0	0	0	+	+	0
Chaetomium **sp.**	0	+	0	0	0	+	+	0
Diatrypaceae	0	+	0	0	0	+	-	+
Phaeosphaeriaceae	0	+	0	0	0	+	0	+
Leptosphaeria-**like**	+*	-*	0	+	+*	-*	+	-
Ophiobolus **sp.**	+*	-*	0	0	+*	-*	0	0

|								
Paraphaeosphaeria michotii	0	+	0	0	0	+	+	0
Pleospora-like	+*	-*	0	0	+*	-*	+	-
Xylariaceae	-	+	-	+	+	-	-	+
Sporormiella 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	+	+*	-*	=	+
Venturia-like	0	+	0	+	0	+	+	0
Sordaria sp.	0	0	0	0	0	0	0	0
Herbothidia 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 significative ($p < 0.05$).

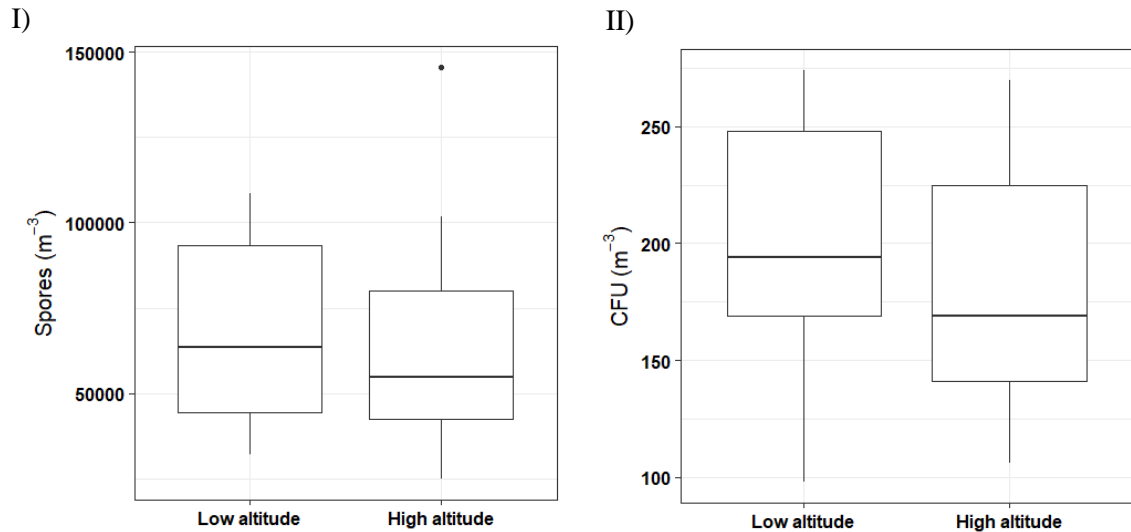I)   II)
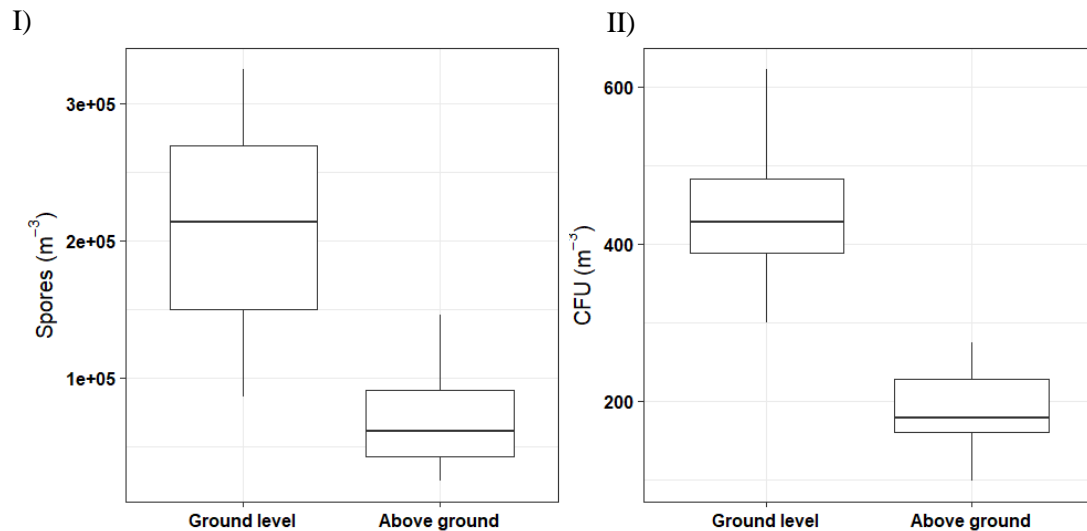
315

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.

I)   II)
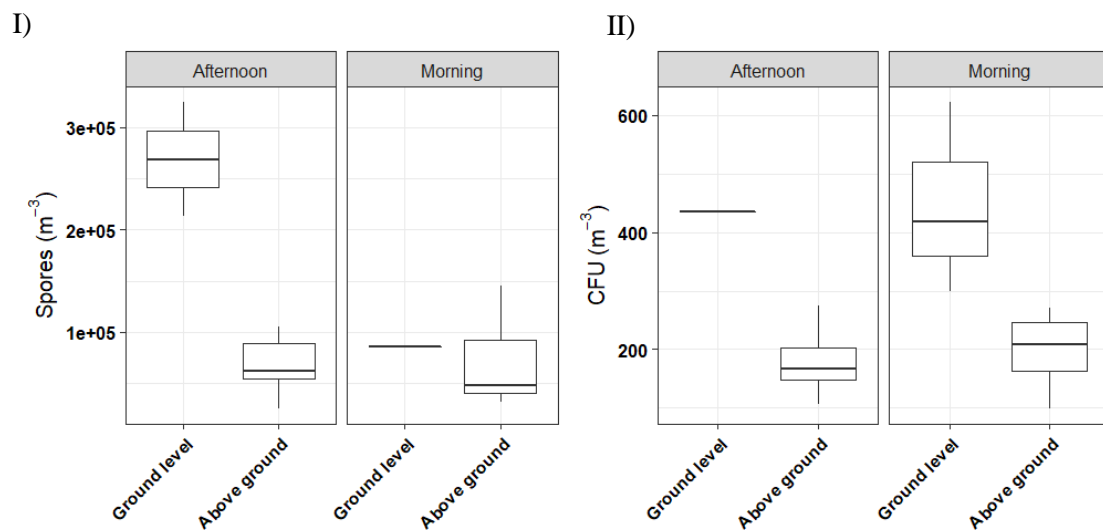

321
322   **Fig. 4** Boxplot of the concentration at ground level and above ground considering the Winter
323   Campaign and different periods of day (morning and afternoon). I) average fungal spore
324   concentration (spores/m³) from portable Burkard (Afternoon: W=20, p<0.05); II) average fungi
325   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).

I)   II)

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

345

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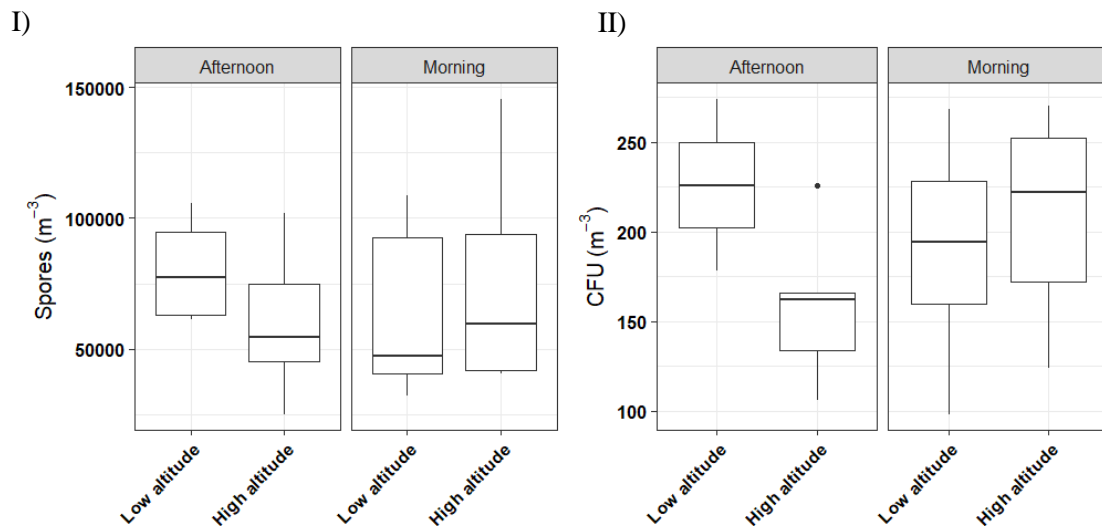
346
347   **Fig. 6** Boxplot of the concentration at low altitude (up to 211 m), and high altitude (>211m)
348   considering the Winter Campaign and the morning and afternoon period. I) average fungal spore
349   concentration (spores/m³) from portable Burkard; II) average fungi colony forming unit (CFU/m³)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

354

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 analyzis was performed considering the Burkard concentrations. Average concentration of *Cladosporium* sp. and *Pen/Asp*-like at ground level was 24,672 (±22,567) spores/m³ and 26,858 (±41,353) spores/m³ respectively, and above ground was 2,021 (±1,700) spores/m³ and 2,321 (±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.
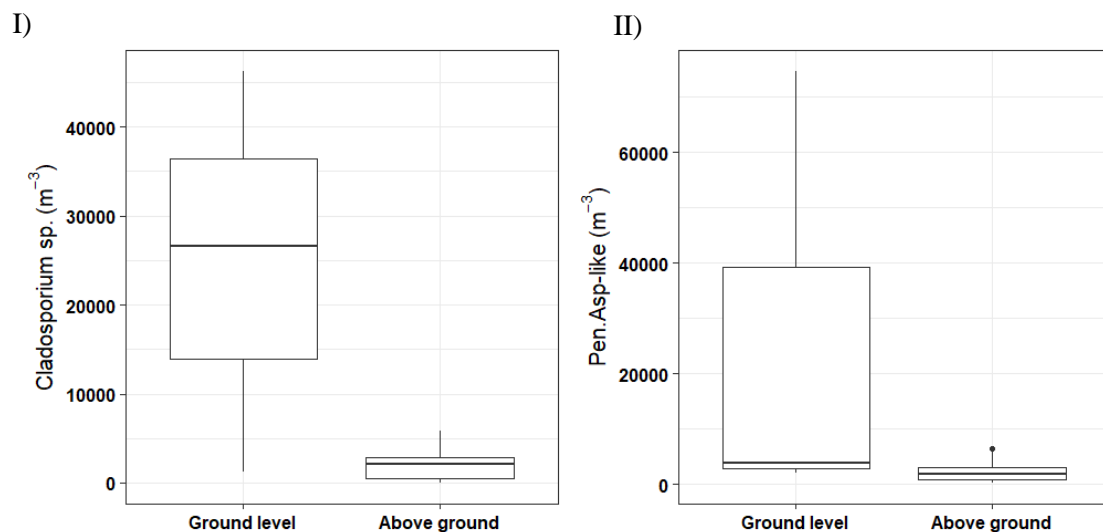
Fig. 7 Boxplot of the concentration at ground level and above ground from portable Burkard and considering the Winter Campaign. I) average *Cladosporium* sp. concentration (spores/m³); II) average *Pen/Asp*-like concentration (spores/m³); For *Cladosporium* sp. and *Pen/Asp*-like the ground level had a n=3, and for above ground a n=21.
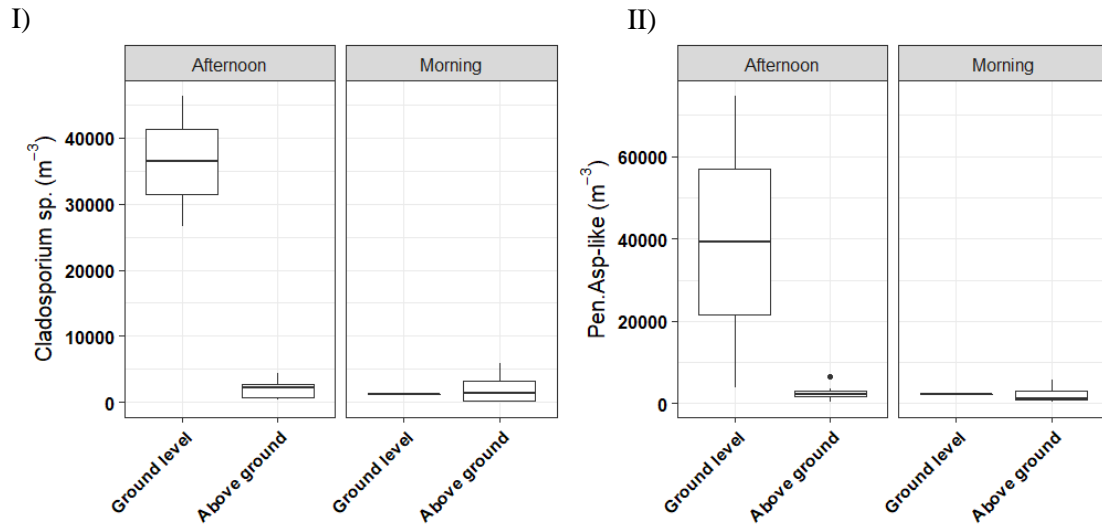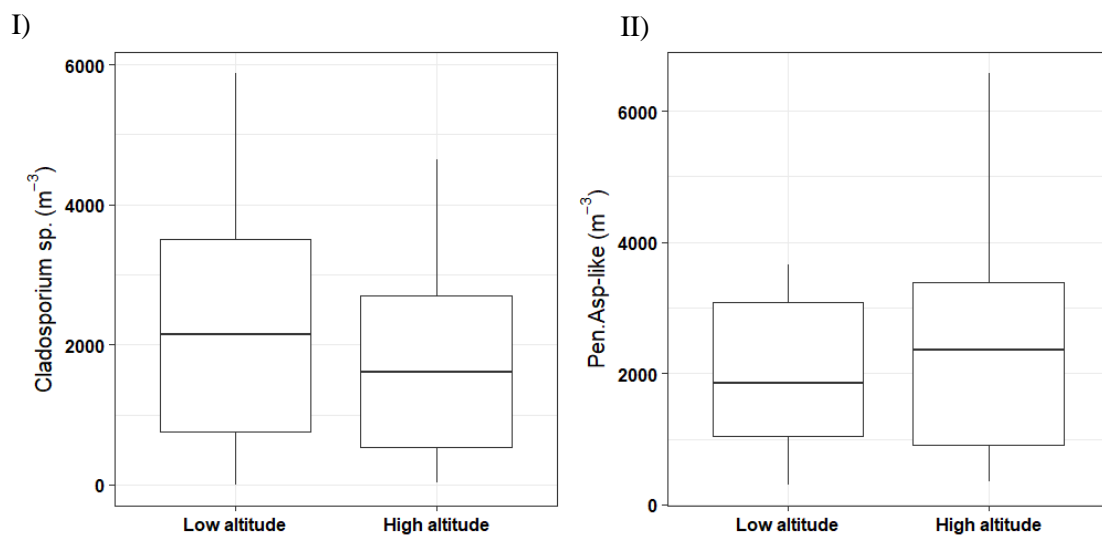
Fig. 8 Boxplot of the concentration at ground level and above ground from portable Burkard considering the Winter Campaign and different periods of day (morning and afternoon). I) average *Cladosporium* sp. concentration (spores/m³) (Afternoon: W=20, p<0.05); II) average *Pen/Asp*-like concentration (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.

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.

15

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

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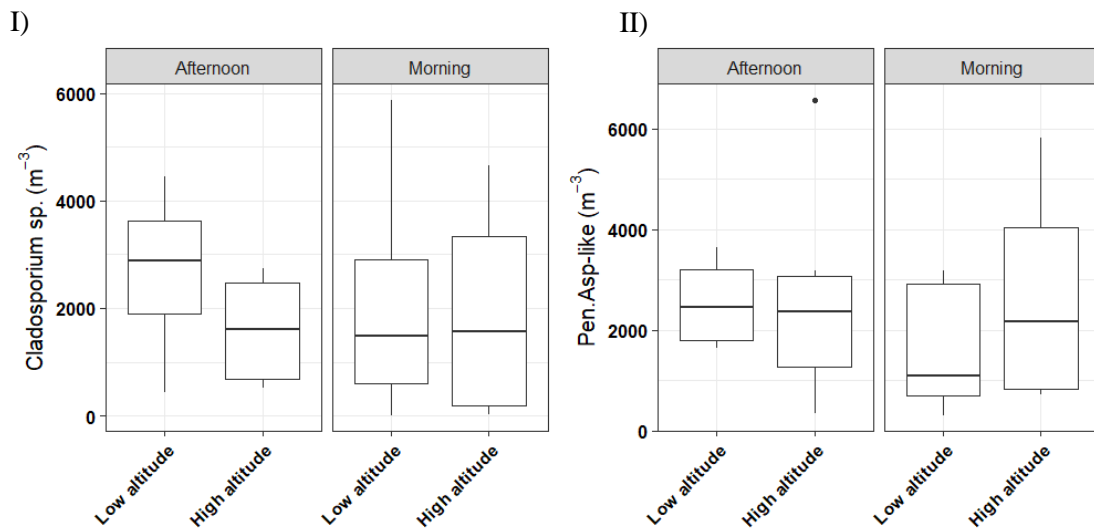
Fig. 10 Boxplot of the concentration at low altitude (up to 211 m), and high altitude (>211 m) considering the Winter Campaign and the morning and afternoon period. I) average *Cladosporium* sp. (spores/m³) from portable Burkard; II) average *Pen/Asp*-like (spores/m³) from portable Burkard. For *Cladosporium*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

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

4. Discussion

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

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

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

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

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

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

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722

7.   Supplementary information

724
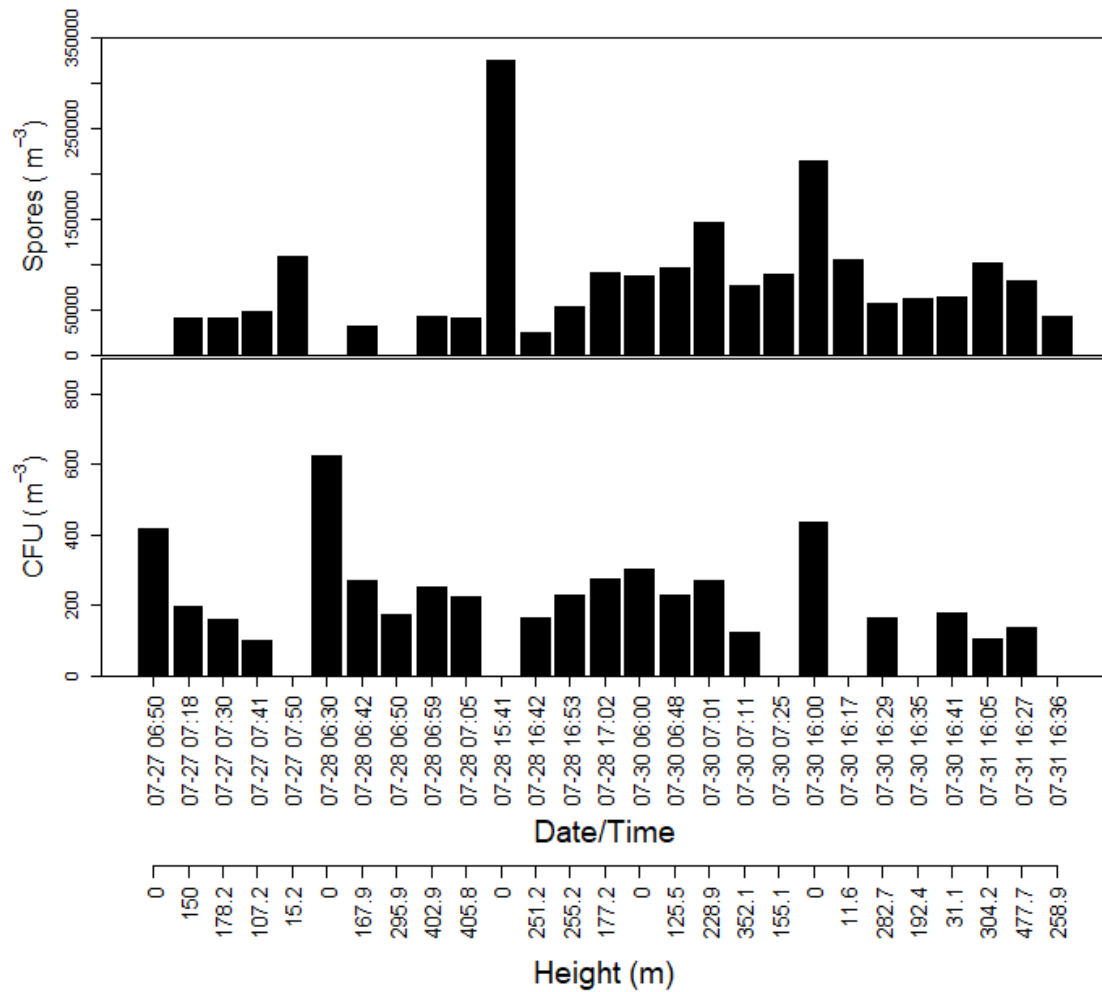
Fig. SI 1 Temporal variation and respective sampling heights considering the Winter Campaign. I) Fungal spore concentration (spores/m³) from portable Burkard; II) Fungi colony forming unit (CFU/m³) from MAS100.

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

Date	Height (m)	Spores/m³	CFU/m³
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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