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

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

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

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 -8ºC to -10ºC 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.,

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

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

2. Methodology

Sampling site

Both sampling sites are based on Arceburgo town, at Minas Gerais State of Brazil, as seen in Fig. 1, showing the different land use such as coffee crops, sugar cane crops, pasture and natural reservations. One of the sampling sites was located at southwest of the Arceburgo city, in a coffee farm close to a pasture (21º23'36.37" S and 46º55'16.48" W) and the other was in the northwest of the Arceburgo city, were only the sample from day 30.07.19 was collected (21º18'48.25" S and 46º50'40.44'' W). The ground samplings were collected immediately before the balloon flight for bioaerosol measurements.

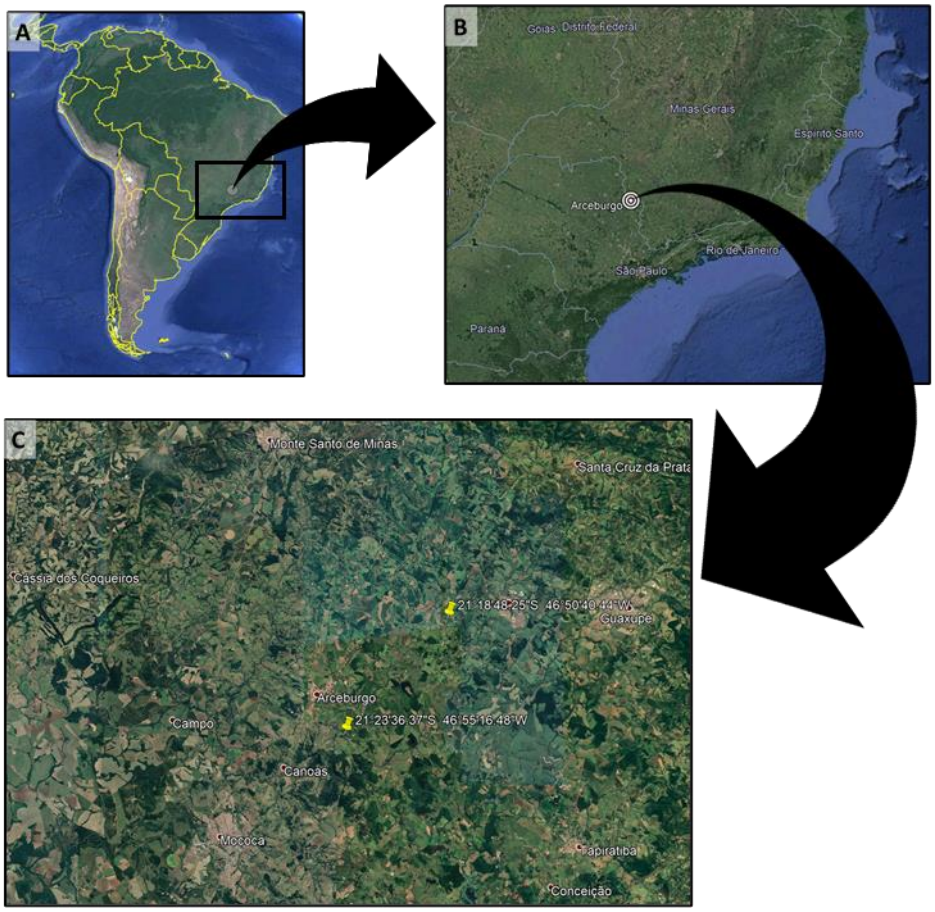

Fig. 1 Map with the sampling sites. A: South America; B: Southeast of Brazil; C: Region of the sampling site centered at the 21º23'36.37" S and 46º55' 16.48" W, and at 21º18'48.25" S and 46º50'40.44" W (yellow markers), nearby Arceburgo City (21º 20' 35" S and 46º56'21.56" W, ~716 m asl), where the balloon flights started (Source: Google Earth).

Sampling methodology

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

Sampling devices

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

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

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

Sampling period

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

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

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

Statistics

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

Auxiliary meteorological data were obtained at hourly resolution from the global ERA5 reanalyzes of the European Centre for Medium-Range Weather Forecasts (ECMWF). Specifically, height of the boundary layer (BL) has been extracted from the ERA5 data on single-levels (SL) dataset (Hersbach et al., 2018a), while relative humidity (RH) and geopotential height were obtained from the ERA5 data on pressure-levels (PL) dataset (Hersbach et al., 2018b). Both datasets report data from 1979 to the present at an hourly time resolution and with horizontal resolution of 0.25°x0.25°. The closest pixel to the Arceburgo sampling area was selected for both datasets (-21.40° latitude, -46.93° longitude). The main difference between the two datasets is the kind of variables found in them and their vertical distribution: geopotential and relative humidity are reported at pressure levels in the atmosphere, specifically on 37 pressure levels from 1 to 1000 hPa. The actual height above ground of the relative humidity data was obtained in two steps: first geopotential was converted to geopotential height by dividing it by Earth's gravitational acceleration (obtaining the height above sea level of the corresponding pressure level) and finally by subtracting the ground elevation from the geopotential height in order to obtain the height above ground level of the pressure levels. Ground elevation for the ERA5 pixel's coordinates (666 m) was obtained from the Terra Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) Global Digital Elevation Model (GDEM) Version 3 (NASA et al., 2019). After this transformation was made, only the levels with height above grounds compatible to the balloon flights were selected which, for both measurement campaigns, where the levels between 925 and 700 hPa, corresponding to height above grounds ranging between roughly 100 and 2500

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

3. Results

3.1. Overall description of fungal analyzis

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

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

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

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

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

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

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

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

3.2. Meteorological data

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

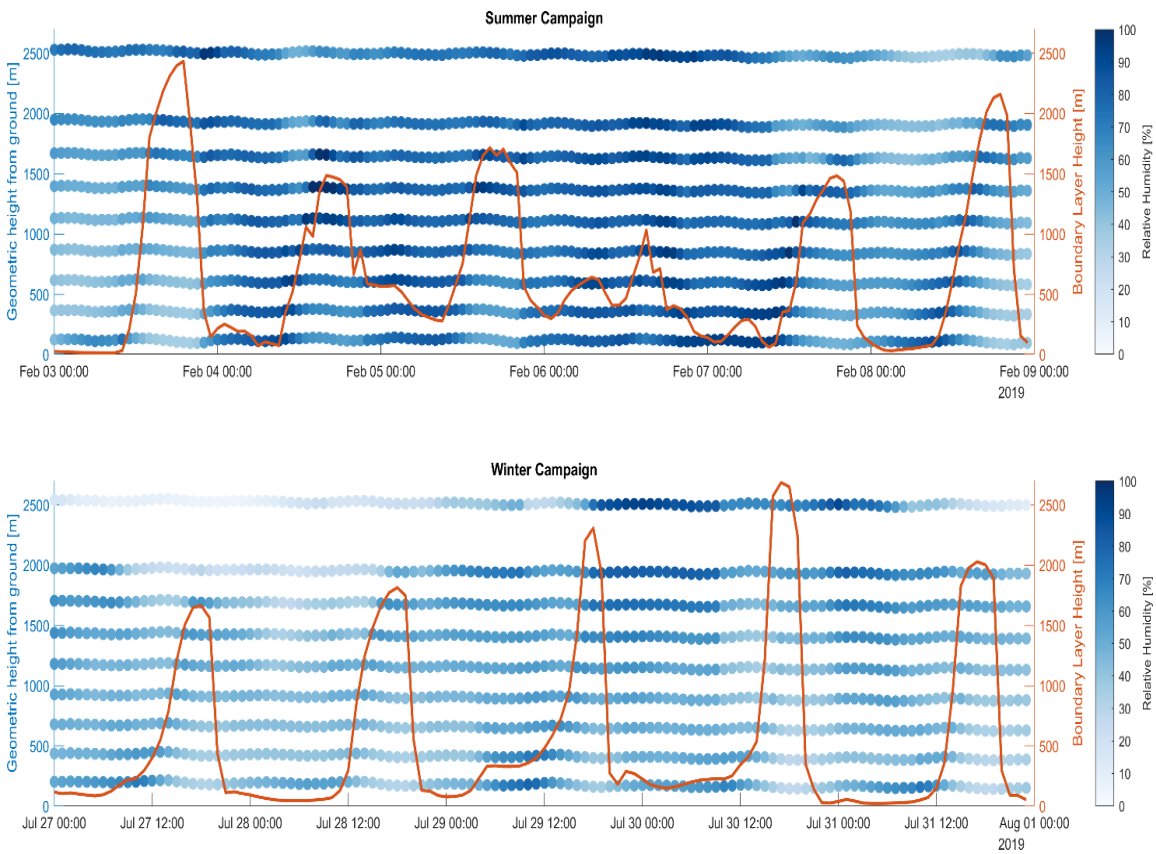

Fig 2 Relative humidity and boundary layer (BL) heights for the two sampling Campaigns. Position of the colored dots indicates height above ground (left y-axis), while the intensity of the blue color of the dots indicates RH percentage (colorbar). The orange line indicates the height of the BL (right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

3.3. Overall Fungal concentration in different altitudes

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

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

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	+*	-*	+*	-*	+	-	+	-

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

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

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

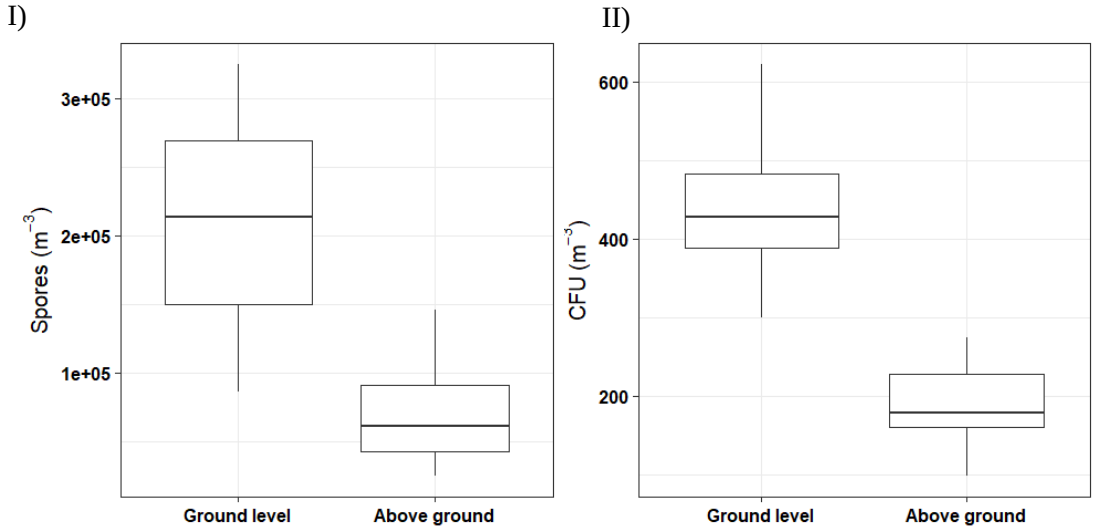

Fig. 3 Boxplot of the concentration at ground level and above ground considering the Winter Campaign. I) average fungal spore concentration (spores/m³) from portable Burkard (W = 56, p <0.05); II) average fungi colony forming unit (CFU/m³) from MAS100 (W = 68, p <0.05). For Burkard, the ground level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

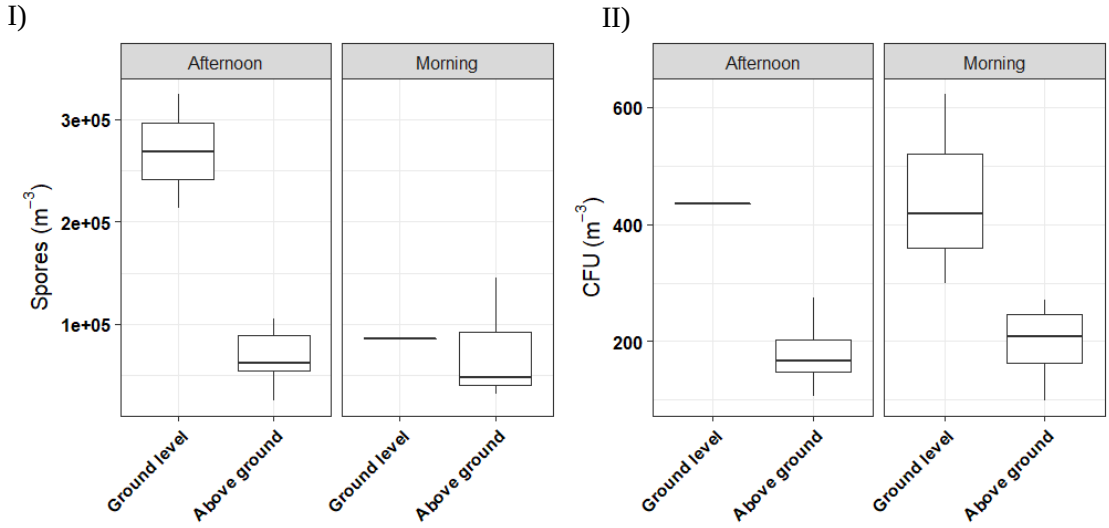

Fig. 4 Boxplot of the concentration at ground level and above ground considering the Winter Campaign and different periods of day (morning and afternoon). I) average fungal spore concentration (spores/m³) from portable Burkard (Afternoon: W=20, p<0.05); II) average fungi colony forming unit (CFU/m³)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

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

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

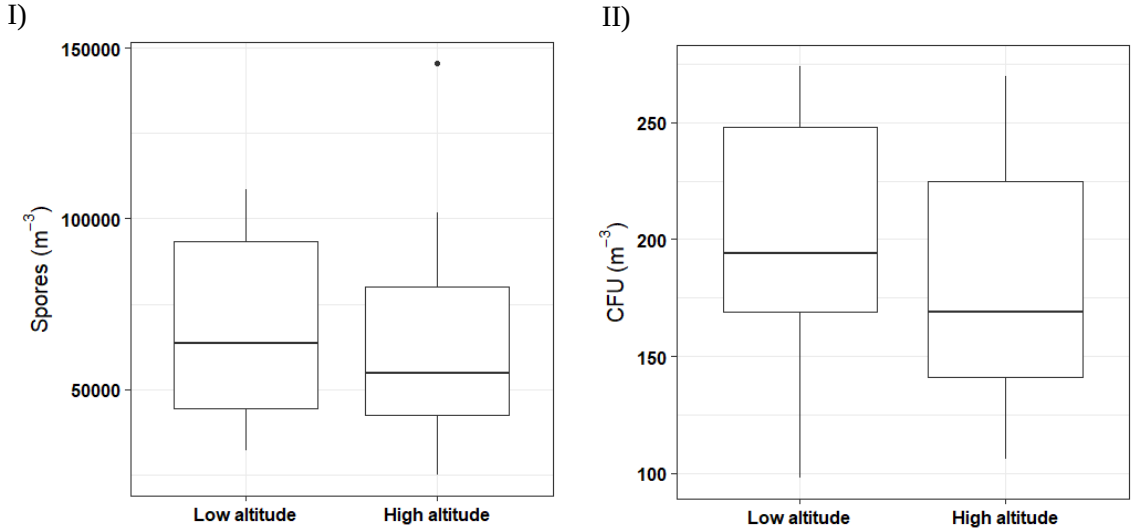

Fig. 5 Boxplot of the concentration at low altitude (up to 211 m) and high altitude (>211 m) considering the Winter Campaign. I) average fungal spore concentration (spores/m³) from portable Burkard; II) average fungi colony forming unit (CFU/m³) from MAS100. For Burkard, the low altitude had a n=11,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

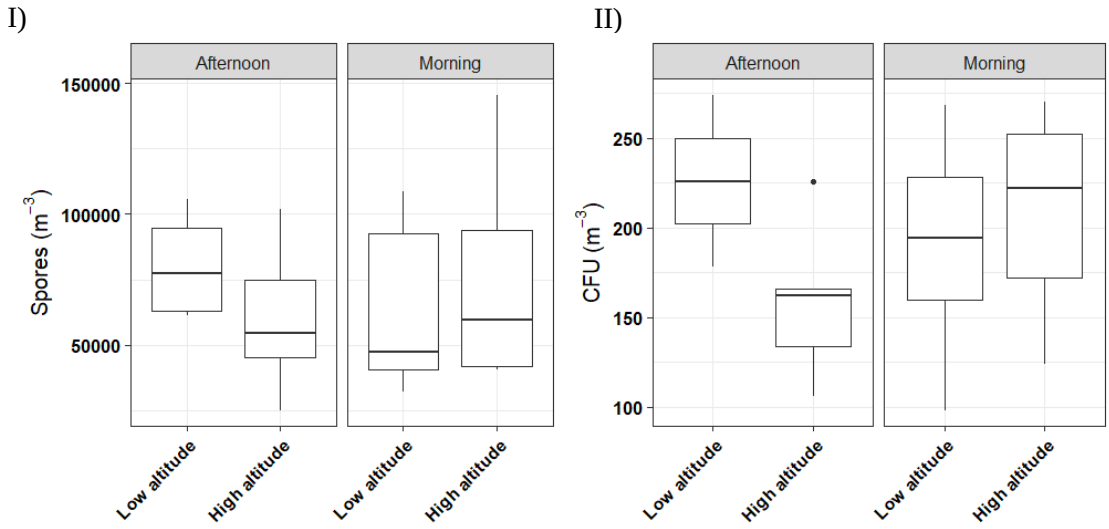

Fig. 6 Boxplot of the concentration at low altitude (up to 211 m), and high altitude (>211m) considering the Winter Campaign and the morning and afternoon period. I) average fungal spore concentration (spores/m³) from portable Burkard; II) average fungi colony forming unit (CFU/m³) from MAS100. For Burkard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

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.

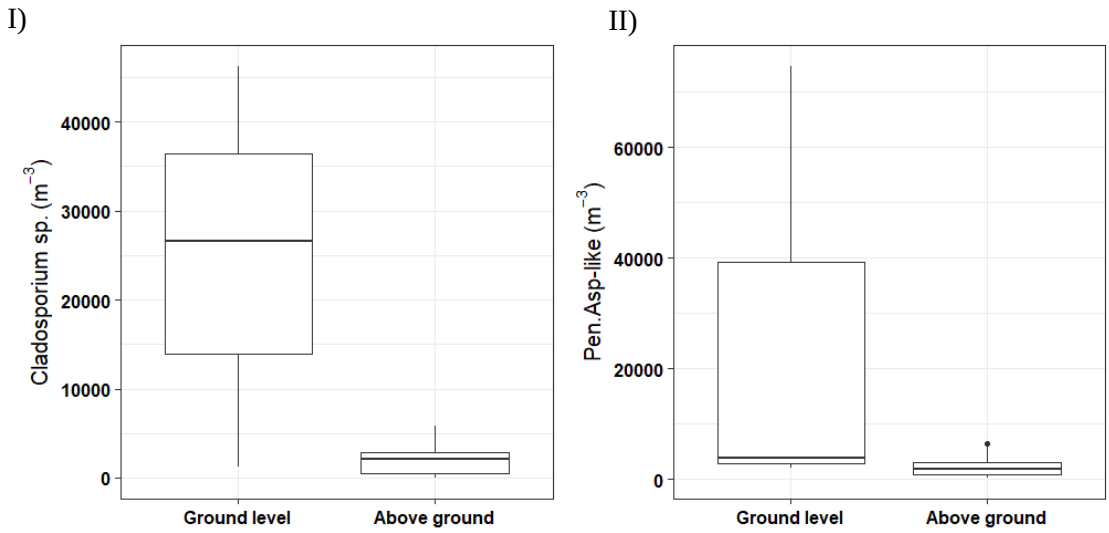

Fig. 7 Boxplot of the concentration at ground level and above ground from portable Burkard and considering the Winter Campaign. I) average *Cladosporium* sp. concentration (spores/m³); II) average *Pen/Asp*-like concentration (spores/m³); For *Cladosporium* sp. and *Pen/Asp*-like the ground level had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

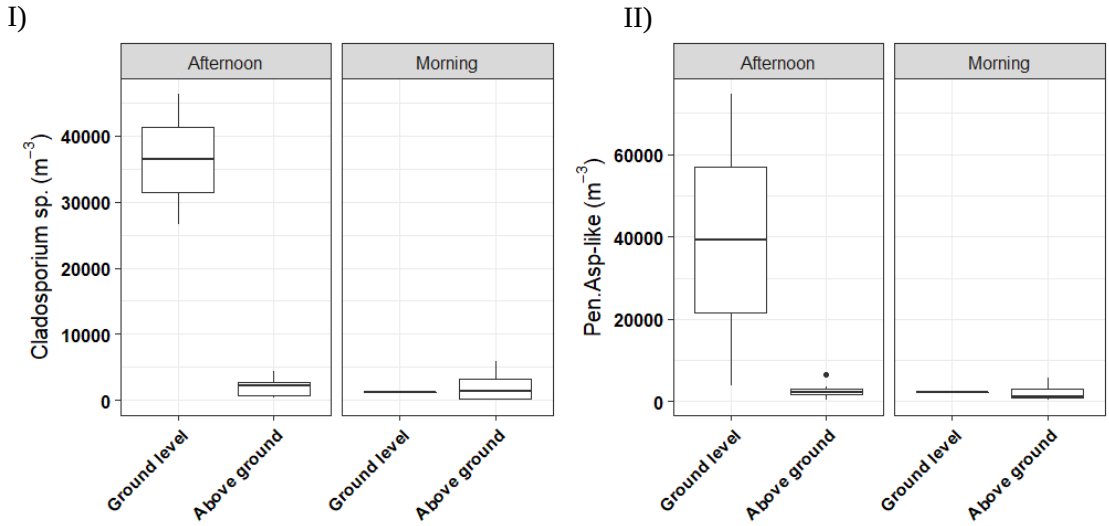

Fig. 8 Boxplot of the concentration at ground level and above ground from portable Burkard considering the Winter Campaign and different periods of day (morning and afternoon). I) average *Cladosporium* sp. concentration (spores/m³) (Afternoon: W=20, $p<0.05$); II) average *Pen/Asp*-like concentration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

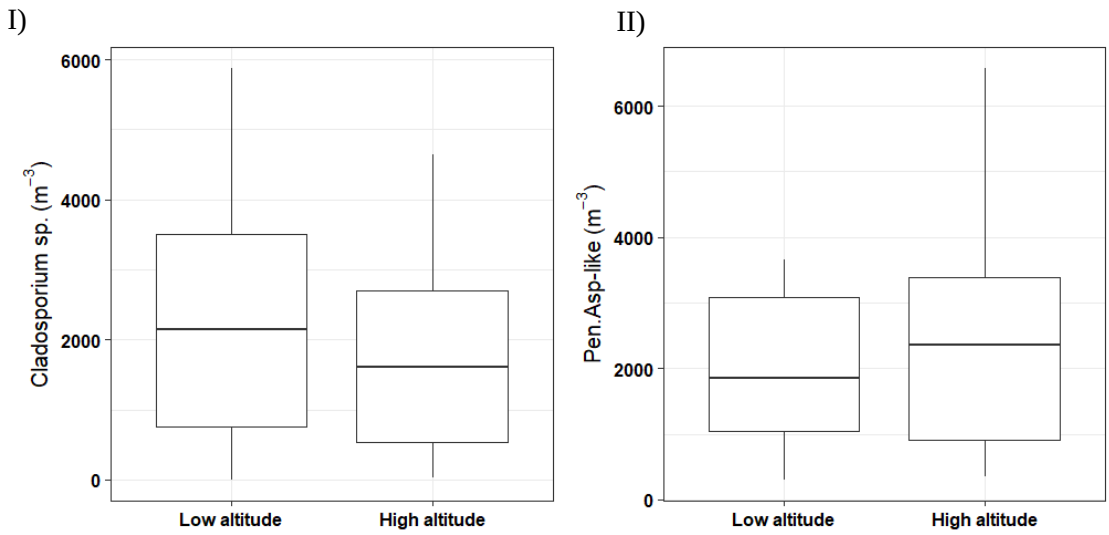

Fig. 9 Boxplot of the concentration at low altitude (up to 211 m) and high altitude (>211 m) considering the Winter Campaign. I) average *Cladosporium* sp. concentration (spores/m³) from portable Burkard; II) average *Pen/Asp*-like concentration (spores/m³) from portable Burkard. For *Cladosporium* sp.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

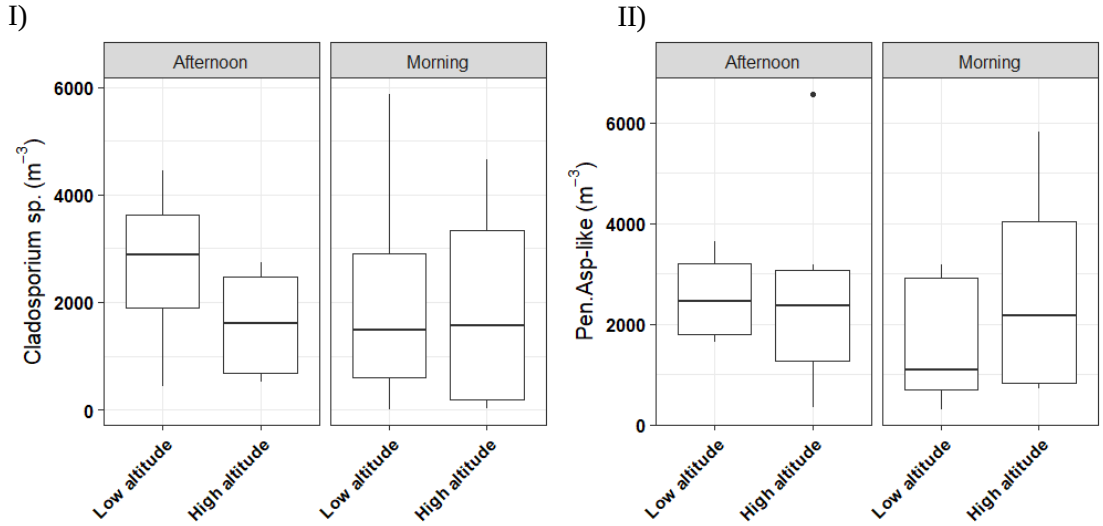

Fig. 10 Boxplot of the concentration at low altitude (up to 211 m), and high altitude (>211 m) considering the Winter Campaign and the morning and afternoon period. I) average *Cladosporium* sp. (spores/m³) from portable Burkard; II) average *Pen/Asp*-like (spores/m³) from portable Burkard. For *Cladosporium*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

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

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

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

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

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

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

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

5. Conclusion

Bioaerosols are increasing their relevance in many studies around the world, but they are not yet fully understood. This work aimed to analyze fungal samples at different altitudes as well 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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7. Supplementary information

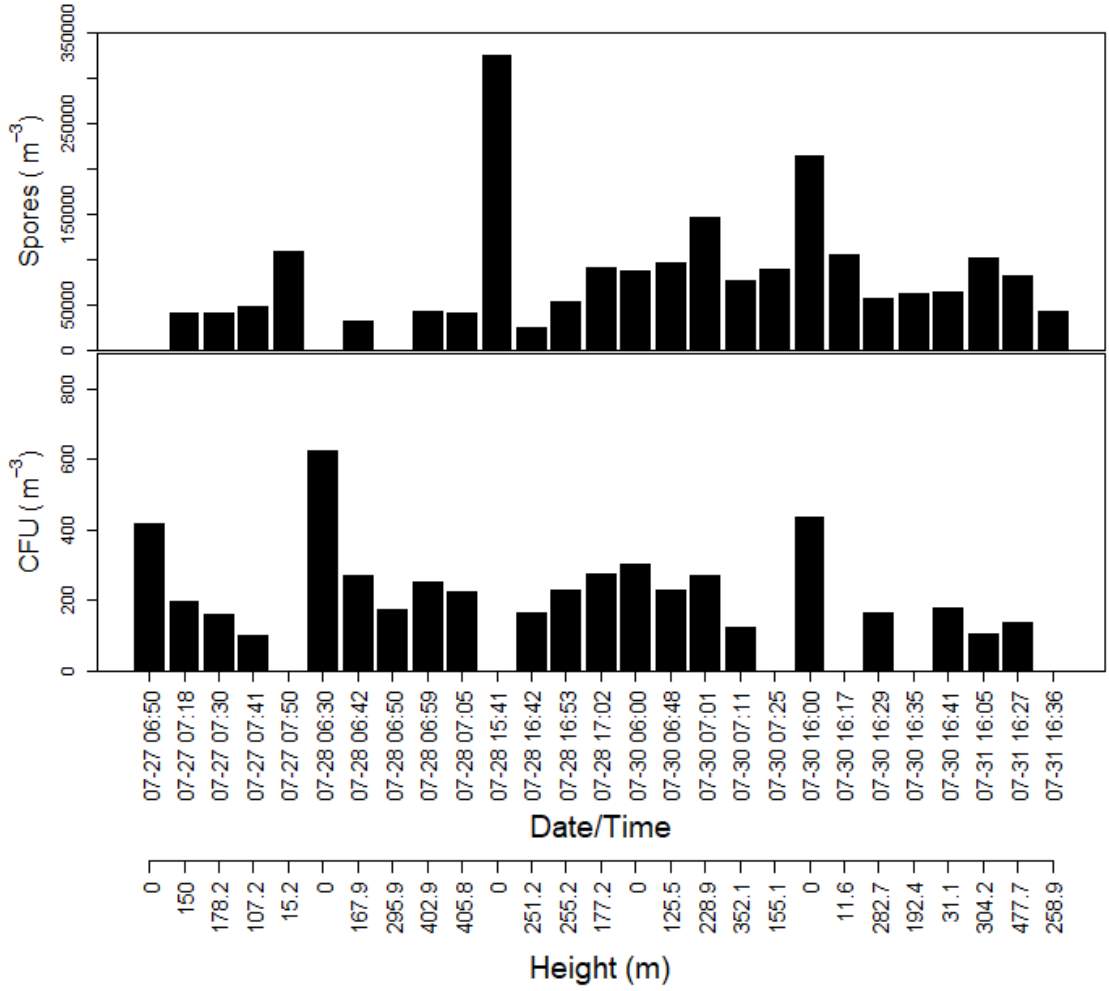

Fig. SI 1 Temporal variation and respective sampling heights considering the Winter Campaign. I) Fungal spore concentration (spores/m³) from portable Burkard; II) Fungi colony forming unit (CFU/m³) from MAS100.

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/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