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

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

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

Introduction:

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

Most true fungi (i.e., Eumycota) disperse via the atmosphere (Golan & Pringle, 2016), which has implications for their presence in the planetary boundary layer of the atmosphere – the lowest part of the atmosphere influenced by the planetary surface. The dispersion of some fungi can also be triggered by precipitation (Löbs et al., 2020), which would be highlighted in areas that have high pluviosity and water availability as is the case of the Brazilian Atlantic Forest (Dalagnol et al., 2022). Meteorological events, therefore, may contribute to fungal emissions (or liberation) to the atmosphere (Grinn-Grofoń et al., 2019; Fagodiya et al., 2022). Since several fungal genera, for instance *Cladosporium*, *Fusarium*, and *Penicillium*, impact on cloud, rain, snow, and hail formation by means of ice nucleation activity (Fröhlich-Nowoisky et al., 2012; Kunert et al., 2019), understanding whether fungal PBAPs present any stratification patterns that could (partially) explain these processes would enhance our comprehension of climate regulation (Šantl-Temkiv et al., 2022). Nonetheless, sampling at high altitudes can be difficult due to the low retrieval of microbial material (Šantl-Temkiv et al., 2020; Tignat-Perrier et al., 2020). Additionally, given the stochasticity of rainfall events, particularly in the light of climate change (Dalagnol et al., 2022), there is a need to investigate the drivers behind fungal vertical profiles in the planetary boundary layer, such as rainfall events. This would be important not only to verify the effects that fungal PBAPs have on cloud formation and rain facilitation, but also to understand how these meteorological events contribute to the dispersion of airborne fungi.

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

Material and Methods

Area of Study and Experimental Design

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

We planned the campaign during the rainy season as we were interested in the effects of precipitation on fungal spore concentration in the atmosphere. To characterise fungal PBAPs vertical profiles, we performed four consecutive flights (between 8th and 11th of March 2022) with a hot-air balloon. Each flight lasted *c.* 1 hour. Flights were performed in early mornings (06:00 – 08:00 local time) since this was the only period in which the balloon could be safely flown. Early morning corresponds to the period of the atmospheric boundary layer growth and this phenomenon can influence the vertical distribution of PBAPs. This is a limitation of the study that was taken into account and appropriately discussed in the following sections. Flights reached the maximum height of *c.* 800 m from ground level, and since we were not able to collect replicate-samples above 300 m, we show data for 0-300 m only. There was a rain event on March 10th just before our third flight, so the analysis is divided into prior to and after the rainfall, with two flights in each period. According to data collated by the meteorological stations in the region (COOXUPÉ, 2022 – available at: cooxupe.com.br), on the 10th of March, the total amount of cumulative rainfall for the region was 17 mm, with an average temperature of 23.3 °C and an average air humidity of 73.7%.

Rainfall Estimation using IMERG

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

ERA5/ECMWF Meteorological Data

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

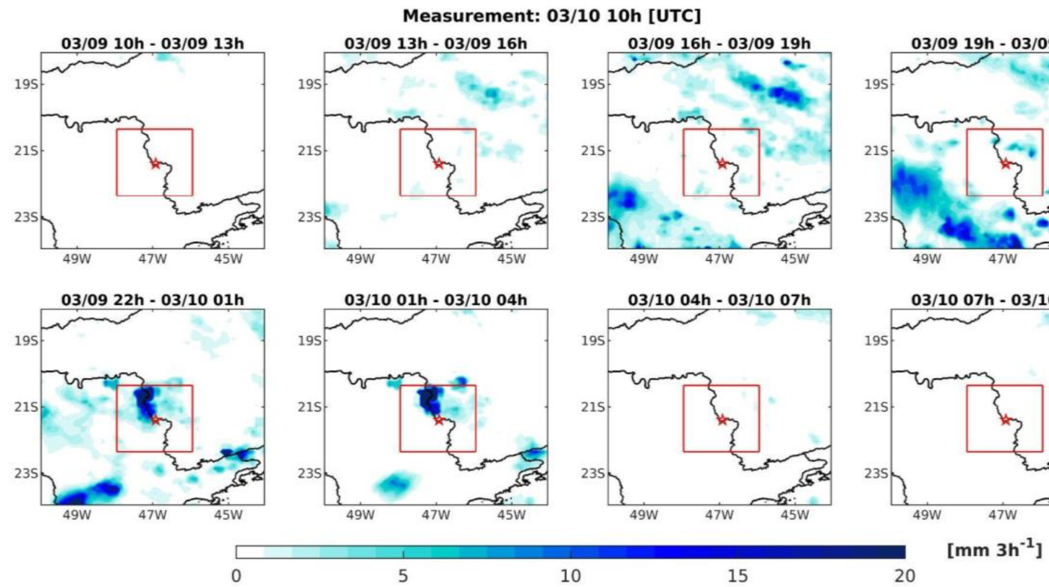

Figure 1 – Three-hourly IMERG spatial precipitation pattern in the 24 hours preceding the measurement on the 10th of March at 10:00 UTC. The two bottom quadrants on the left, referring to 01:00-04:00 UTC, show an estimated precipitation of 15-20 mm just before the third flight. The red box represents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

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

We are aware of the limitations of models in simulating boundary layer height. Nevertheless, there was no available radiosonde close enough to the sampling region that could be used in this study. ERA5 has, thus, been chosen to obtain boundary layer information following a recent paper by Guo et al. (2021). The paper made a near-global comparison between daytime boundary layer height from various reanalysis products and measurements made by radiosonde. Even if the comparison was done only at synoptic times (00:00 and 12:00 UTC), ERA5 was shown to be the reanalysis having the smaller bias and the highest positive correlations relative to radiosondes (Guo et al., 2021).

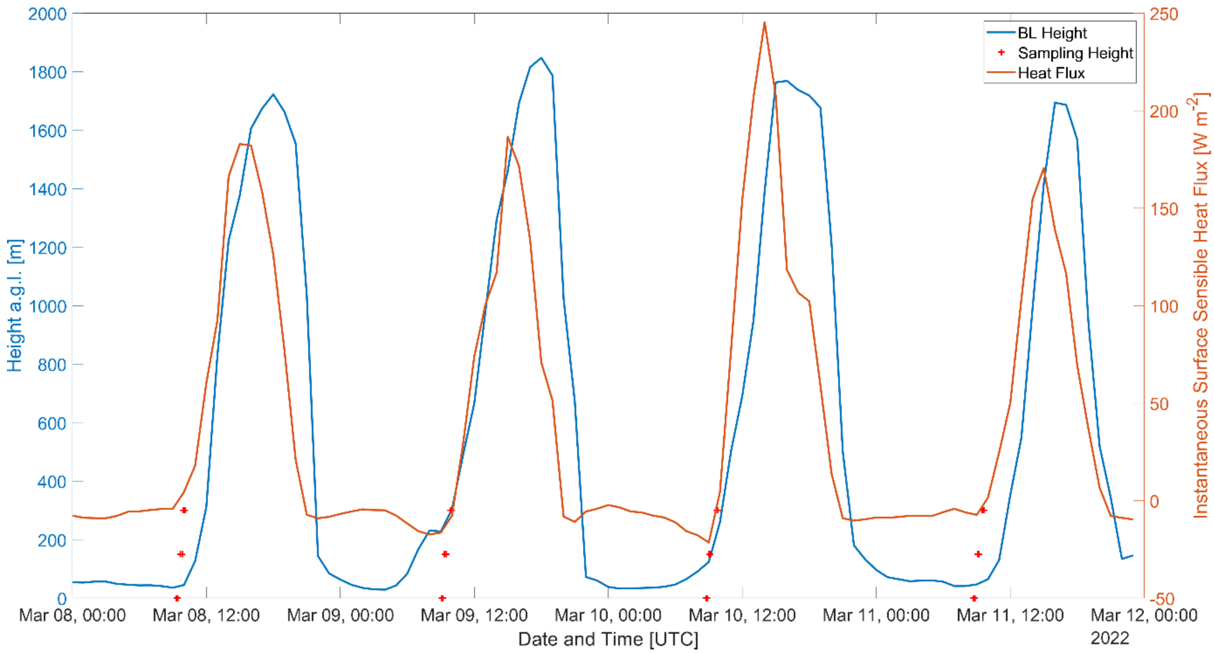

Figure 2 – Trend of meteorological data from the ERA5 reanalysis during the sampling period. On the left y-axis it is shown the height above ground of the boundary layer (BL, light blue line) and the PBAP samplings (red crosses), while on the right y-axis it is shown the value of the instantaneous surface sensible heat flux (orange line).

Fungi Sampling using the Portable Burkard Air Sampler

We collected *in situ* airborne fungal PBAPs using a portable Burkard air sampler (Burkard Manufacturing Co., Hertfordshire, UK), as per Emygdio et al. (2022). Three sampling altitudes were examined: 0 m or ground level, 150 m, and 300 m above ground level. At the ground level, the instrument was placed at 0.3 m over a small table (Supplementary Figure 1A). To sample at 150 and 300 m, the portable Burkard air sampler was mounted and stabilised in the passenger basket of the hot-air balloon (Supplementary Figure 1B). Once the desired sampling height was reached, the hot-air balloon floated steadily during sample collection. The variation of flight height was calculated to be *c.* 10% of the desired one, so sampling heights were 150 ± 15 m (i.e., 135-165 m) and 300 ± 30 m (i.e., 270-330 m).

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

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

Fungi Sampling using the Microbial Air Monitoring System (MAS100)

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

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

Statistical Analysis

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

Results

Portable Burkard Air Sampler Results

The total concentration of fungal spores varied with altitude, and it was much bigger at the ground level (118,420 fungal spores m^{-3} or 76% of all spores counted), followed by the 150 m layer (19,700 spores m^{-3} or 17%), and the 300 m layer (8,280 spores m^{-3} or 7%) (Table 1). A significant interaction between the rainfall event and sampling heights was found ($F_{(2,18)} = 14.26$; $P < 0.001$). We observed a pronounced reduction in the concentration of fungal spores at 150 m (95% CI = 0.455, 1.066) and 300 m (95% CI = 0.324, 0.935) after the rainfall. This resulted in a 4- to 5-fold decrease in fungal spore concentrations caused by rainfall (Table 1). However, at the ground level, as the concentration of fungal spores showed only a minor reduction (*c.* 15%), the total concentration of fungal spores after the rainfall was not significantly different in comparison to before the rain ($F_{(2,18)}$ = 14.26; P = 0.799; 95% CI = -0.191, 0.419). Thus, most spores (90%) after the rainfall event were found at the ground level.

Table 1 – Concentration (spores m^{-3}) and percentage of *Cladosporium* sp. and *Aspergillus/Penicillium*-like fungal spores at the region of Arceburgo city, Minas Gerais state, Southeast Brazil. Note: "All Data" refers to all data collected in the whole experiment, whereas "Before Rainfall" or "After Rainfall" refers to data sampled prior or after the rain that occurred at the dawn of the third day of the fieldwork campaign (10^{th} of March 2022) and before the third hot-air balloon flight.

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

Considering only the two main fungi groups in the studied region, *Cladosporium* sp. dominated all the three sampling heights examined, representing more than 80% of all spores counted, whilst *Asp/Pen*-like accounted for nearly 20%. Prior to the rain, *Cladosporium* sp. were 3- to 4-fold more abundant than *Asp/Pen*-like at all heights (ground level, $F_{(2,18)}$ = 5.168; P = 0.017; 95% CI = 0.411, 0.882; 150 m, 95% CI = 0.251, 0.722; and 300 m, 95% CI = 0.228, 0.699; Figure 3). After the rainfall, at the ground level, *Cladosporium* sp. were 5-fold more abundant ($F_{(2,18)}$ = 5.168; P = 0.017; 95% CI = 0.606, 1.08) than *Asp/Pen*-like (Figure 3C). Nevertheless, for the 150 m and 300 m, these ratios were reduced to 2-fold ($F_{(2,18)}$ = 5.168; P = 0.017; 95% CI = 0.076, 0.547; Figure 3B) and 2.5-fold ($F_{(2,18)}$ = 5.168; P = 0.017; 95% CI = 0.188, 0.659; Figure 3A), respectively. These shifts in fungal concentration driven by the rain were paralleled by the proportion of spores sampled at the different heights (Table 1).

MAS100 Results

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

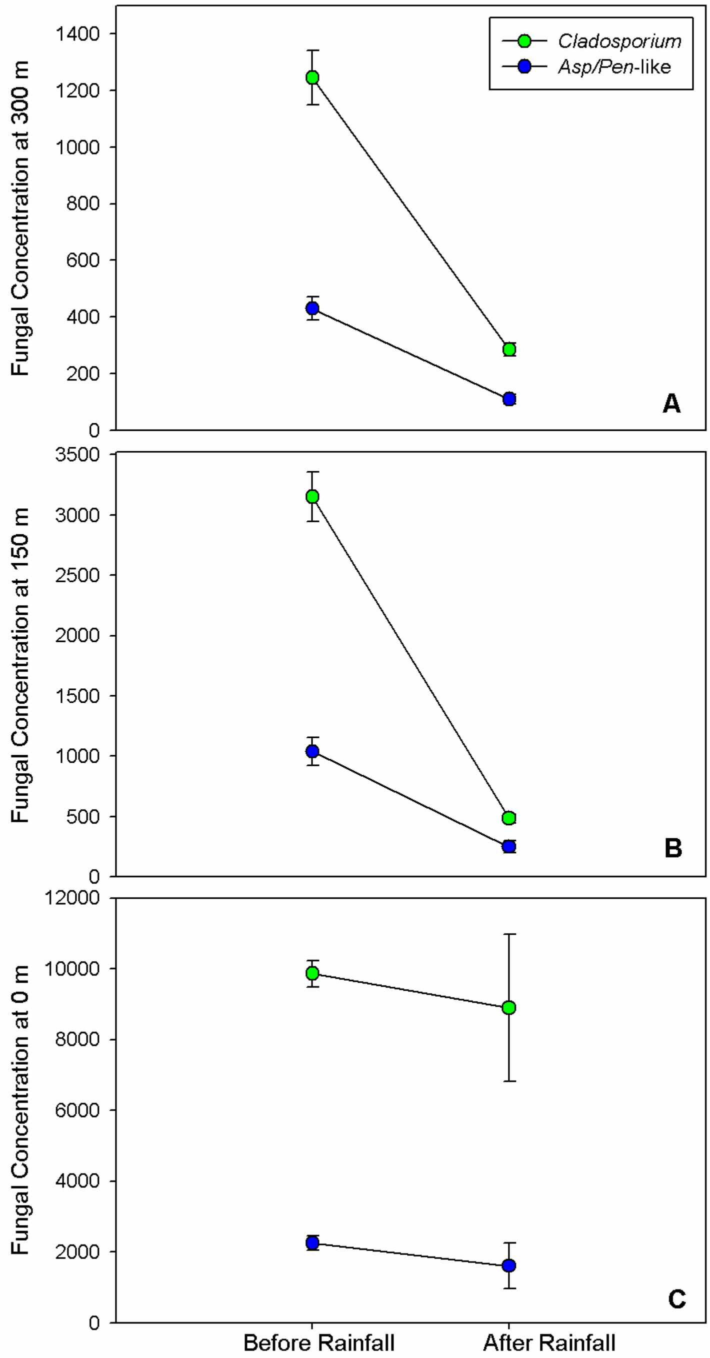

Figure 3 – Concentration (spores m^{-3}) of *Cladosporium* sp. and *Aspergillus/Penicillium*-like spores at (A) 300 m, (B) 150 m, and (C) 0 m (i.e., ground level) sampled at the region of Arceburgo city, Minas Gerais state, Southeast Brazil (n = 4; mean ± SE; non-transformed data). Legend: “Before Rainfall”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

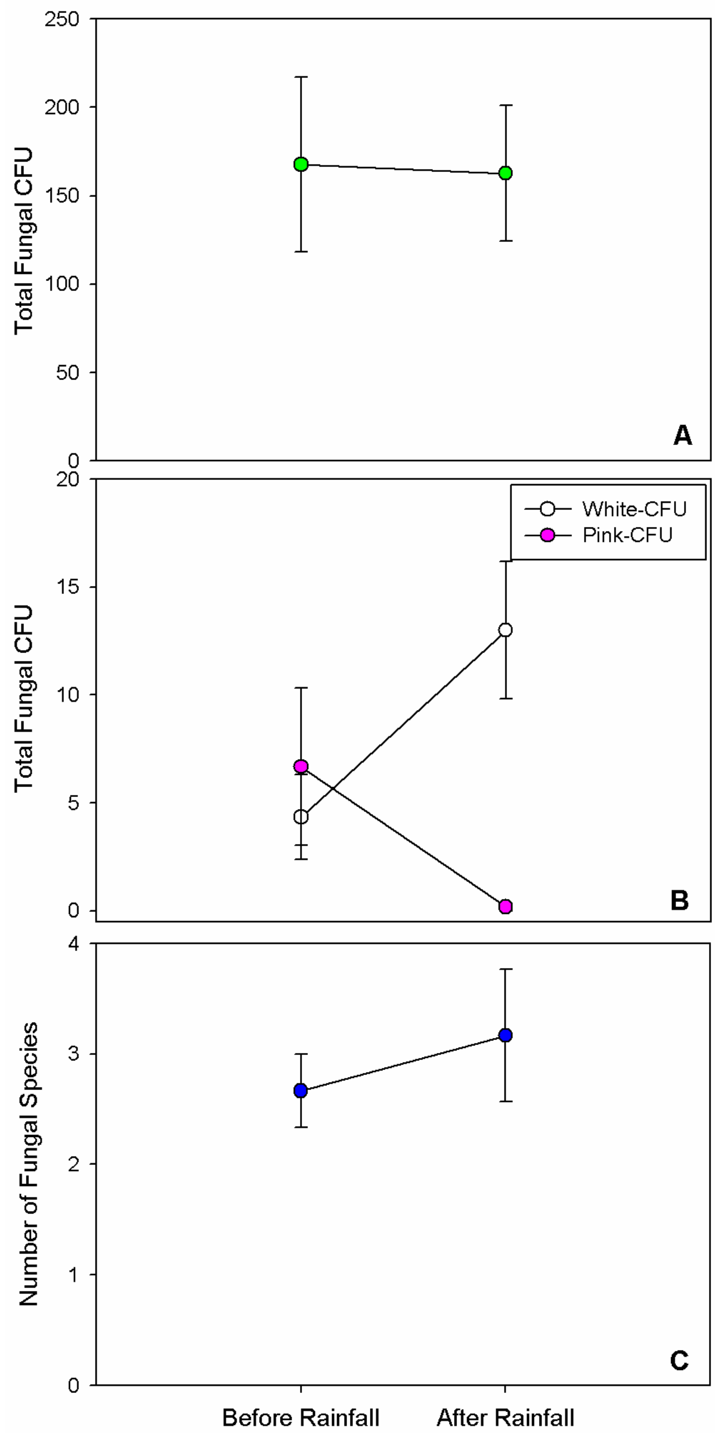

Figure 4 – Total fungal CFU (A), White-CFU and Pink-CFU (B), and number of fungal species (C) sampled at the region of Arceburgo city, Minas Gerais state, Southeast Brazil (n = 6; mean ± SE; non-transformed data). Legend: “Before Rainfall” or “After Rainfall” refers to data sampled prior or after the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

Correlations between Fungal PBAPs and Sampling Heights

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

Discussion

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

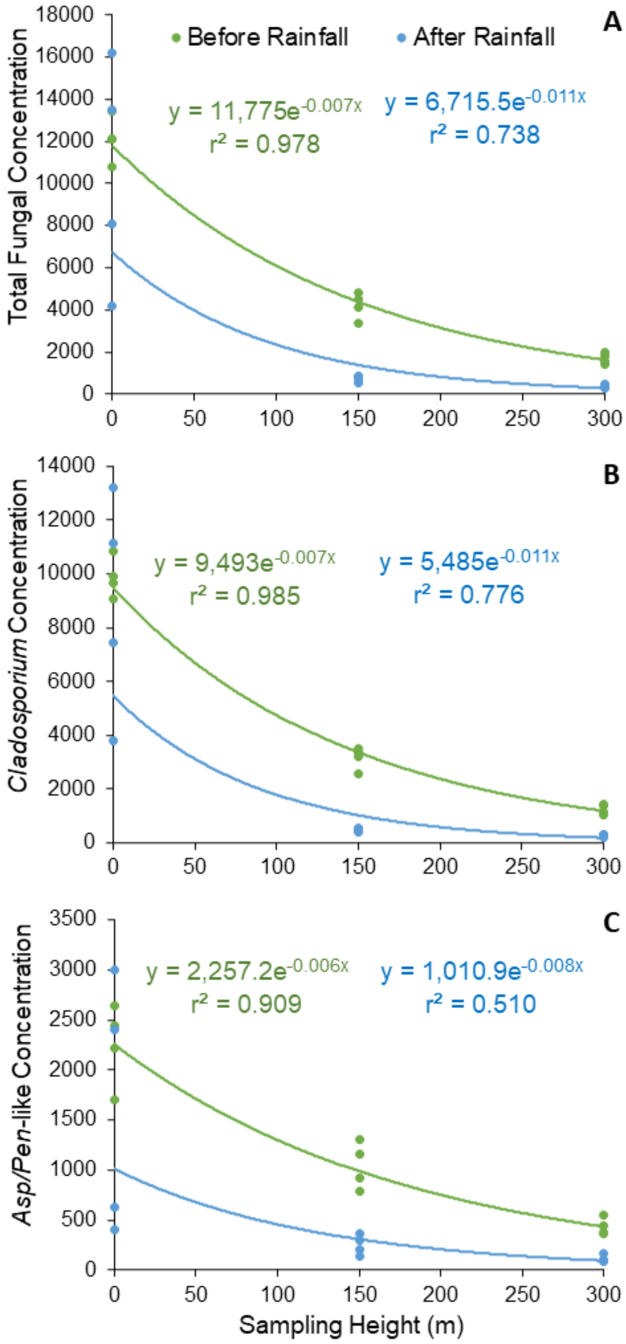

Figure 5 – Regression analyses between (A) the total fungal concentration (spores m^{-3}), (B) *Cladosporium* sp. concentration (spores m^{-3}), and (C) *Aspergillus/Penicillium*-like concentration (spores m^{-3}) with sampling height (0-300 m) at the region of Arceburgo city, Minas Gerais state, Southeast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

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

Whilst height was shown to be a factor structuring fungal communities in the atmosphere both based on modelling and experimental studies (Sesartic et al., 2013; Els et al., 2019a; 2019b; Tignat-Perrier et al., 2020; Emygdio et al., 2022), we demonstrate that rainfall is an even stronger factor driving the removal of fungal PBAPs from the atmosphere (Yue et al., 2016; Rathnayake et al., 2017). This confirms our third hypothesis that the concentration of fungal spores present in the atmosphere would be reduced once rainfall occurs. After the rain, the number of *Cladosporium* sp. and *Asp/Pen*-like spores were reduced from higher layers. This indicates that rainfall drives the wet removal of fungal PBAPs from the atmosphere to the ground, reducing their concentration at the studied sampling heights (Jensen et al., 2022). Nevertheless, Huffman et al. (2013) have registered high concentrations of bioaerosol during and after rain onset, which were linked to forest canopies that have triggered PBAPs emissions. Since the region where the samples were collected features a mixed land-use pattern including coffee plantations, pasture for cattle, and sugar-cane crops interspersed with Atlantic Forest fragments (Emygdio et al., 2022), differences in how rainfall events impact PBAPs fluxes may have to consider the land-use context, warranting more research on this. Furthermore, the time of day in which the sampling was done may influence the results obtained in this study, as well as the meteorological conditions prior to and after the rain, so results should be taken with caution. In fact, early morning flights might have happened above the atmospheric boundary layer height, thus affecting the representativeness of fungi vertical gradients.

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

Conclusions

Taking altogether, the results presented in our study demonstrate the differential effects exerted by rainfall and altitude from ground level on fungal PBAPs, showing a use for the fungal gradient at different sampling height to compare with a process induced by rain. In general, an increase in height and rain occurrence lead to reductions in the concentration of fungi present in the atmosphere in the study area within the Brazilian Atlantic Forest biome. The higher prevalence of *Cladosporium* sp. at all sampling heights demonstrates the ubiquity of this fungal species, which could be considered as a proxy for other fungal PBAPs in future studies aiming to investigate cloud and precipitation formation by such microorganisms. This has further implications for research on climate regulation, such as considering the land-use context in where sampling is taken, as well as collating other types of data (e.g., PBAPs fluxes), which warrants more investigation on the role of fungal PBAPs worldwide.

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

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

Time	**Height (m)**	**Fungi Genera/Species**
Before Rainfall	0	*Cladosporium* sp.; *Fusarium oxysporum*; Sterile Mycelium; Yeasts
	150	*Cladosporium* sp.; *Fusarium equisiti*; *Penicillium* sp.; Yeasts
	300	*Cladosporium* sp.; *Curvularia* sp.; *Fusarium chlamydosporum*; *Penicillium* sp.; Sterile Mycelium
After Rainfall	0	*Alternaria* sp.; *Cladosporium* sp.; *Curvularia* sp.; *Fusarium* sp.
	150	*Alternaria* sp.; *Cladosporium* sp.; *Fusarium equisiti*; *Penicillium* sp.; Sterile Mycelium; Yeasts
	300	*Alternaria* sp.; *Cladosporium* sp.; *Fusarium equisiti*; *Penicillium* sp.; Sterile Mycelium

Morphotype-Species	**Presence in Samples**	**Frequency (%)**
Cladosporium	11	92
Fusarium	6	50
Penicillium	5	42
Alternaria	4	33
Sterile Mycelium	4	33
Yeasts	3	25
Curvularia	2	17

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

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