



Local meteorological conditions, shape and desiccation influence dispersal capabilities for airborne microorganisms

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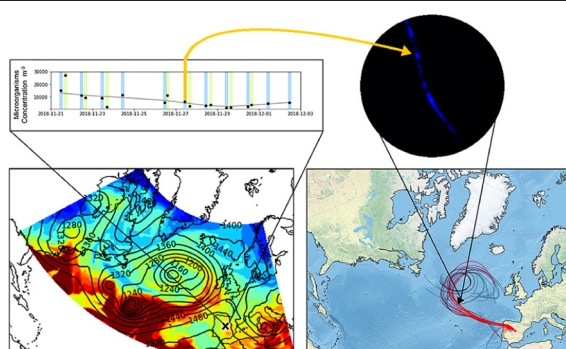
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HIGHLIGHTS

- Abundance of airborne microorganisms changes abruptly in short time
- Local meteorology, as a whole, affects abundance of airborne microorganisms
- Higher abundance of airborne microorganisms in cyclonic period than in anti-cyclonic
- New dispersion simulations of airborne bacteria considering shape and desiccation
- Non-spherical microorganisms up to 400 μm can disperse long distance

GRAPHICAL ABSTRACT



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ABSTRACT

The atmosphere plays an important role in the dispersal of microorganisms, as well as in the connectivity of most of the planet's ecosystems. In recent decades, interest in microbial diversity and dispersion in the atmosphere has increased due to its importance in various fields. However, there are few studies on the abundance of airborne microorganisms and the factors, such as meteorology, that affect their distribution. Likewise, the physical-mathematical models attempting to reproduce their possible origins also require integrating some biological features. We collected airborne microorganisms under different meteorological conditions at a sampling station over a 12-day period to expand the knowledge about abundance of airborne microorganisms, their relationship with atmospheric conditions and their possible origins with a biological perspective. Total abundance and size distribution of microorganisms were measured in all samples using epifluorescence techniques. Their possible origins were estimated using refined mathematical simulation models of the air masses back-trajectories considering dry deposition. Our results showed microbial abundance values similar to those found in temperate regions over land surface. In our contribution we report a clear relationship between the abundance and, considered as a whole, local meteorological conditions. Despite most of the captured particles were small spherical microorganisms (diameter < 20 μm), large filamentous microorganisms, surprisingly up to 400 μm , were also found. We demonstrate the possibility that these large microorganisms can have their origin at long distances, showing thus probability of remarkable long dispersal, without ruling out a nearby origin, when their equivalent spherical diameter (ESD) and drying capacity are considered.

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1. Introduction

The atmosphere, despite its crucial role as a means of microbial transport across the planet, is the least known biome on Earth (Uetake et al., 2020; Cáliz et al., 2018; Wilkinson et al., 2012). It is a habitat that houses airborne microorganisms which are of great importance for various fields of study. In relation to epidemiology, there are numerous cases of human diseases associated with them. Some examples are asthma associated with fungal spores (Grinn-Gofroni and Strzelczak, 2013), diseases caused by endotoxins of some bacteria (Mueller-Annealing et al., 2004) or serious respiratory diseases caused by viruses (Setti et al., 2020). However, airborne microorganisms have an impact not only on human health, but also in other relevant economic sectors such as phytopathology (Morris et al., 2007), microbial ecology (Monteil et al., 2014) and meteorological and climatological sciences (Fröhlich-Nowoisky et al., 2016; DeLeón-Rodríguez et al., 2013).

Even though it is known that atmosphere is a habitat that houses a high number and diversity of microorganisms, their concentrations per cubic meter of air are low. Most estimations indicate that there are around 10^4 cells m^{-3} of air over the land surface and 10^2 – 10^4 cells m^{-3} of air over the sea (Mayol et al., 2014, 2017; Burrows et al., 2009; Bauer et al., 2002). In some specific events, concentrations of microorganisms can reach larger values, of up to 5.9×10^6 cells m^{-3} of air (DeLeón-Rodríguez et al., 2013). Previous works on microbial atmospheric dispersion have mostly focused on biodiversity of airborne microorganisms, their space-time variation and their relationship with their possible origins (Uetake et al., 2020; Archer et al., 2019; Tignat-Perrier et al., 2019; Šantl-Temkiv et al., 2018; Bowers et al., 2013), but few are the studies about the variation in the abundance of airborne microorganisms, which remains secondary, and their relationship with atmospheric conditions (Dong et al., 2016; Burrows et al., 2009; Tong and Lighthart, 2000).

Knowing these abundance thresholds and the causes of their variation are of great importance of many fields of study. On the one hand, they are useful in ecology to establish and predict the level of risk against the arrival of invasive species into vulnerable ecosystems due to climate change. On the other hand, this knowledge would allow to avoid and control pests that could spoil entire crops or to avoid and control the transmission of infectious diseases in the livestock industry or among human populations (Weil et al., 2017; Meola et al., 2015; Smith, 2013). However, the variability in the abundance of airborne microorganisms is not well understood. Some authors suggested that the abundance may correlate with some meteorological variables, as wind speed (Cáliz et al., 2018; DeLeón-Rodríguez et al., 2013; Mouli et al., 2005), but other environmental characteristics (e.g., temperature, humidity or precipitation) do not appear to be related with concentrations in a direct way (Dong et al., 2016; Yue et al., 2016; Harrison et al., 2005; Mouli et al., 2005; Tong and Lighthart, 2000). In general terms, the relationship between airborne microorganisms (diversity and abundance) and meteorological conditions is not well characterized yet (Fröhlich-Nowoisky et al., 2016; Burrows et al., 2009).

Thanks to atmospheric air masses, biological particles, a term that includes prokaryotic cells, small eukaryotes, fungal spores, pollen and acellular structures such as viruses, can be transported and deposited thousands of kilometres away from their origin. This long travelling is favoured by the long residence time of microorganisms in the air due to their small size (Griffin et al., 2017; Mayol et al., 2017; Wilkinson et al., 2012; Wilkinson, 2001). However, not all microorganisms are susceptible to being suspended or transported through the atmosphere for long distances. To establish which are the physical limits of microorganisms to be transported by air, physical-mathematical models have been developed reproduce the trajectories followed by the air masses that transport them. However, some diversity aerobiology studies did not consider dry deposition. Under this unrealistic scenario, the biological particles may remain for extremely long periods of time in suspension and be dispersed large distances regardless their size. When dry

deposition is incorporated in the models, the average size of the spherical particles susceptible to remain in suspension corresponds to diameters of 1–4 μm (Burrows et al., 2009). Larger airborne microorganisms are considered unable of travelling far away due to deposition process which essentially depends on the microorganisms' size and density. Recent models suggest that microorganisms of about 20 μm present a low probability of being dispersed and those with diameter over 60 μm cannot be dispersed (Wilkinson et al., 2012; Wilkinson, 2001).

The aim of the present study is to investigate the variation in the abundance of airborne microorganisms of different sizes and its possible relationship with local meteorological conditions. We conducted an experiment in which we collected samples of airborne microorganisms in a period of 12 consecutive days in a fixed sampling location with changing meteorological conditions. The highest abundance of airborne microorganisms corresponded to microorganisms that are considered small in size and therefore can have distant sources. We also unexpectedly found large filamentous microorganisms for which current models are not able to estimate their origin. We investigated their dispersion capacity and possible origins by refining the current physical-mathematical models from a more biological perspective considering, for the first time, equivalent spherical diameter (ESD) and their drying capacity.

Airborne microorganisms are part of the atmospheric aerosols. The transport of any particle in the air depends on the size and density, and therefore biological particles are subjected to the same principles. However, unlike many inorganic particles, these characteristics in biological particles can change and this makes the dispersion capacity higher. The current models consider, for the sake of simplicity in the modelling of back-trajectory of air masses, that the airborne microorganisms are spherical. However, most of the microorganisms have filamentous shapes, as cyanobacteria. In this case, to consider the physical forces that tend to sediment the particles we propose for the first time that they must be characterized in terms of their equivalent spherical diameter (ESD), which gives a diameter much smaller than the length of elongated particles. Despite the application of the term ESD to airborne non-biological particles (Chen and Fryrear, 2001; Yu and Standish, 1993), until the study presented here, ESD had not been applied to real biological samples to determine their dispersion capacity. Therefore, these non-spherical particles behave, in terms of sedimentation, as much smaller particles. Concurrently, models do not consider inherent characteristics of many airborne microorganisms that can affect their dispersion capability, like the drying capacity, among others. In the study presented here, drying capacity of microorganisms has been incorporated in the dispersion models. Since several microorganisms, like cyanobacteria or many green algae species, have been observed to be able to reach water concentrations as low as 2% of the full hydration, which would be equivalent to a loss of 98% of its density, and rehydrate fairly quickly afterwards when humidity is high enough (Holzinger and Karsten, 2013; Potts, 1999). In those ecosystems where atmospheric relative humidity can be extremely low, mainly hot deserts or polar ecosystems (Antony et al., 2016; Crits-Christoph et al., 2016), a decrease in density may provide to microorganisms a higher range of dispersion since the dry deposition process would be low. This increased dispersion capacity would be of great importance in the study of invasive species or for example in the study of the colonization of new ice-free areas that had been covered for thousands of years, in a context of climate change (Weil et al., 2017).

2. Materials and methods

2.1. Sample collection

A total of 19 air samples were collected between 21 November and 2 December 2018 (Table S1), at the Universidad Autónoma de Madrid campus site, 20 km north of Madrid, Spain (40° 54' 30" N, 3° 69' 16" W), located outside the city. This area is surrounded by protected

Mediterranean forest but urban influence cannot be dismissed. The sampling instrument was deployed on a tripod at 1.3 m above the roof surface of a building at a height of ca. 15 m, to minimize possible influence from very near-surface sources, and far from any obstacle (Fig. S1.A).

Air sampling was carried out using a commercially available cyclonic collector (Coriolis-Δ, Bertin Technologies; [Carvalho et al., 2008](#)) (Fig. S1. B). The collection liquid consisted of Mili-Q water containing 0.005% Triton X-100 and was prepared fresh a week before by sterile filtration (0.2 μm) and autoclave at 110 °C for 15 min. We used a negative control based on unopened and sterile collection liquid to ensure the correct filtration and autoclaving of it. For each sampling day, a new sterile collection liquid was used. The air flowed into the sampler at 300 l per minute during 3 h (equivalent to 54 m³). The collection liquid was refilled automatically by the system at 0.4 ml/min, rate sufficient to match the loss of liquid by evaporation and re-aerosolization.

To ensure cleanliness of the entire system, prior to each sampling, all pieces in contact with the sample were autoclaved at 110 °C for 15 min wrapped in aluminium foil and opened when installed in the instrument at the sampling site. Non-autoclavable pieces were cleaned with HCl (10% final concentration). Then, the decontamination protocol with H₂O₂ (30% final concentration) suggested by the Coriolis-Δ manufacturer was followed. To finish decontamination process, the inlet piece was sprayed with ethanol 95% final concentration. Finally, the non-autoclavable pieces were rinsed with freshly prepared 0.2 μm filter-sterilized Mili-Q water to remove these chemical compounds which can affect the viability of the collected microorganisms. Before each sampling, another negative control was collected from the sterile collection liquid, after passing for the complete collection system to ensure the cleanliness of the system. To avoid contamination the researchers used biological protection equipment and the system was programmed to automatically start 2 min after preparation without the proximity of the researchers. Samples were processed immediately after collection to prevent any potential reduction in microorganism's viability ([Mayol et al., 2014](#)).

2.2. Microbial abundance and size distribution

Five millilitre aliquots of sample were fixed with formaldehyde (2% final concentration) for minimum 10 min, stained with 13 μl DAPI (4', 6-diamidino-2-phenylindole) at 0.1 mg ml⁻¹ and subsequently filtered onto black 0.2 μm pore size polycarbonate filters (Millipore). Subsequently, the samples were mounted on microscope slides, with EMOIL-F30CC (Olympus) oil as mounting medium, for microscopy analysis of the microorganisms' abundance.

DAPI-stained samples were examined using Nikon Eclipse 80i epifluorescence microscope, equipped with a mercury lamp and a filter cube containing a 380/40 BP excitation filter, a 400 nm dichromatic mirror and a 485/435 BP emission filter, with a 100× objective. DAPI bound to DNA results in bright blue fluorescence at ~390 nm when excited with 365 nm light, while DAPI bound to other materials appears as non-fluorescent ([Porter and Feig, 1980](#)). Those cells uniformly stained (no clearly defined nucleus) and small size were counted as prokaryotes. Cells presenting clearly defined nucleus or with irregular staining and larger were counted as unicellular eukaryotes ([Sherr et al., 1993](#)). A first morphological identification of eukaryotes such as pluricellular uncertain algae and desmidsiales was carried out ([Bellinger and Sigee, 2014](#)). Likewise, in prokaryotes filamentous cyanobacteria and coccoid forms were distinguished ([Komarek et al., 2014](#)). The observed microorganisms were classified by size into three length ranges: 1–5 μm, 5–20 μm and more than 20 μm. To measure the length of microorganisms, Leica DFC300 FX camera and Leica Application Suite v.3.7.0 program were used.

The counting strategy took into account a Poisson distribution that allows to ensure a good overall precision (< 10% relative standard deviation) even in those samples with very low abundances. For this, a total of 20 aleatory fields were counted.

2.3. Meteorological data

Meteorological data, including pressure, air temperature, rainfall, relative humidity and wind speed and direction, were provided by the State Meteorological Agency (AEMET) with a temporal resolution of 10 min. Data were recorded at Colmenar Viejo automatic weather station (AWS), which is the closest station to the sampling point (at 21.3 km). Since the area of study has not a complex orography, the weather conditions recorded at the AWS are assumed to be representative of the conditions at the sampling point. Boundary layer depth and dissipation have been obtained from the closest grid point of the ERA5 reanalysis at hourly intervals. ERA5 is the last generation reanalysis of the European Centre of Medium Weather Forecast (ECMWF) and it has a horizontal resolution of 30 km. The mean of all meteorological data recorded during the 3 h of each sampling, except for the variable rainfall, which is the result of the sum, can be observed in the Table S2.

ERA5 was also used to characterize synoptic weather conditions. Low-level free air conditions have been analysed using the geopotential height and the equivalent potential temperature (θ_e) at 850 hPa (ca. 1500 m). Geopotential height provides an idea of the cyclonic or anticyclonic conditions while θ_e is a thermodynamic quantity that is conserved in reversible moist adiabatic processes and serves to characterize air masses.

2.4. Statistical methods

To estimate the trend along time of the microorganism abundance we adjusted the non-parametric regression LOWESS (locally weighted scatterplot smoothing) model with bandwidth of 50% of data. We used the Chow test for structural change detection in this series. For changepoint detection in the time series of meteorological data we used the Pettitt non-parametric *U* test. This test is an adaptation of the Mann-Whitney test to detect a shift in the central tendency of a time series. Time series of temperature, relative humidity and height of the boundary layer were previously seasonally adjusted. Both tests allowed us to identify changepoints and provided us the estimate of the time when it occurred. The tests were calculated with the 'strucchange' ([Zeileis et al., 2002](#)) and 'trend' ([Pohlert, 2016](#)) packages of the R software.

2.5. Back-trajectories simulation of the air masses carrying the captured microorganisms

The back-trajectories of the air masses that could transport the collected microorganisms were simulated using the semi-Lagrangian model HYSPLIT (Hybrid Single Particle Lagrangian Integrated Trajectory) ([Stein et al., 2015](#)). Global GDAS meteorological system was used as a data entry model at 0.5 degrees.

At one-hour intervals during the sampling period, nine 5-day back-trajectories were simulated starting from the sampling station. Trajectories were initialized every 0.1 fraction of the boundary layer height from 0.1 to 0.9. These heights were chosen to represent all the spectrum of trajectories into the well mixed layer as it is assumed that particles can be found anywhere into its extension ([Von Engel and Teixeira, 2013](#)). When the trajectory height reached to 0 m above the surface, the model is not reliable and the trajectory was interrupted at that point.

To integrate the dry deposition in the model we calculated the gravitational deposition v_{grav} for small-medium particle sizes (length < 20 μm) as proposed by [Seinfeld and Pandis \(2006\)](#):

$$v_{grav} = \frac{d^2 (\rho_p - \rho_{air}) g C_c}{18\mu}, \quad (1)$$

where d (m) is the diameter of the particle (length was usually the same as diameter in these cases), ρ_p (kg m⁻³) is the density of the particle, ρ_{air} (kg m⁻³) is the density of the air that can be calculated from the ideal gas equation $\rho_{air} = P/RT$ (where P is the pressure (Pa),

R the constant of gases ($\text{J kg}^{-1} \text{K}^{-1}$) and T the temperature of the air (K), μ ($\text{Pa} \cdot \text{s}$) is the dynamic viscosity of the air, g is the gravity constant (9.8 m s^{-2}) and C_c is the Cunningham correction given by:

$$C_c = 1 + \frac{2\lambda}{d} \left(1.257 + 0.4 \exp \left(-0.55 \frac{d}{\lambda} \right) \right), \quad (2)$$

where λ is the air mean free path (m) given by:

$$\lambda = \frac{\mu}{u^*} \sqrt{\frac{\pi}{8\rho_{\text{air}} P}}, \quad (3)$$

where u^* is a numerical factor equal to 0.4987445.

Most observed large particles with length $> 20 \mu\text{m}$ had a filamentous shape, and in this cases the diameter d was defined as the corresponding equivalent spherical diameter (ESD) and calculated with the next equation:

$$\text{ESD} = 2 \times \left(\frac{3}{16} \times L \times D^2 \right)^{1/3}, \quad (4)$$

where L (m) is the length and D (m) is the diameter of the filament. The dimensions of the captured microorganisms are in Table S3.

Due to the multiple possibilities of desiccation and atmosphere humidity that may occur in the airborne microorganism transport, the model was run assuming $\rho_p = 1000 \text{ kg m}^{-3}$ (1 g cm^{-3}) in the non-desiccation scenario (Monteith and Unsworth, 2008) and the three drying scenarios of 25%, 50% and 85% desiccation.

3. Results

3.1. Abundance and size of airborne microorganisms

The total airborne microorganism concentration recorded during the sampling time interval ranged from 1.31×10^3 to 2.69×10^4 microorganisms m^{-3} (averaging 7.17×10^3 microorganisms m^{-3}) (Table S4). Negative controls counts were zero. A decreasing trend was estimated for the series of total abundance of microorganisms with a changepoint on the negative slope in the morning of 27 November (Chow test p -value < 0.05) (Fig. 1; Table S5). Despite the variation in the concentration of microorganisms along the sampling period, the prokaryote concentration remained always higher than eukaryote concentration over time (average 4.6×10^3 prokaryotic m^{-3} and average 2.3×10^3 eukaryotes m^{-3}) (Table S4).

Small size microorganisms (length: $1\text{--}5 \mu\text{m}$) dominated the community and represented 94.92% of total airborne microorganisms, compared to those considered as medium (length: $5\text{--}20 \mu\text{m}$) and large size (length $> 20 \mu\text{m}$), represented by 4.80 and 0.28% of total airborne microorganisms, respectively (Table S4). The presence of large microorganisms with filamentous forms and lengths up to almost $400 \mu\text{m}$, such as filamentous cyanobacteria or eukaryotic algae, stood out in 12 of the 19 samples analysed in this study (Fig. 2, Table S4).

3.2. Local weather conditions

Weather conditions during the sampling period were representative of winter meteorological conditions at the interior of the Iberian Peninsula. From 21 to 26 November, the synoptic setting around Iberian Peninsula was characterized by cyclonic conditions, with a succession of fronts crossing the area of study. Those conditions advected relatively warm, moist and unstable air at the low-level free atmosphere. On 27 November, a ridge with anticyclonic flow developed over the area of study leading a large-scale stability only temporally broken by a weak front that crossed between 29 and 30 November (Video S1).

We did not find significant correlations between individual environmental variables and abundance or size distribution (data not shown). However, statistical evidence of a changepoint near 27 November has

been found for almost all meteorological variables: relative humidity, boundary layer height, dissipation boundary layer, pressure and wind speed variables (Pettitt test p -values $\ll 0.001$). The estimated times of the changepoints are shown in Fig. 1 and Table S5. During the first period characterized by cyclonic circulation, there are several precipitation events related with pressure local minima and increases in wind speed associated with fronts crossing over Madrid (Fig. 1; Table S2). During the second period, weather conditions were more stable and characterized by higher pressure and slow winds (Fig. 1; Table S2). The small local pressure minimum between 29 and 30 November, related with weak precipitation, was associated with a weak frontal cross (Fig. 1; Video S1).

Boundary layer height also responded to the low-level free air conditions. During the first period, the increased wind speed mixed more efficiently the low atmosphere, increasing the height of the boundary layer over 500 m during the day (Fig. 1; Table S2). Just before the changepoint, there was a major cyclonic event that produced a maximum of boundary layer dissipation and height (Fig. 1). During the second period, the stable conditions allowed to a more efficient decoupling of the boundary layer, preventing to exceed 300 m height and descending to less than 100 m in the night (Fig. 1; Table S2). The only exception to those conditions was during the frontal pass on 29–30 November (Fig. 1).

3.3. Back-trajectories transporting the microorganisms

Five-day back-trajectories starting from the boundary layer of the sampling station during the sampling periods have been simulated to analyse the possible origin of the air masses that transported the captured airborne microorganisms (Fig. 3). Results show that airborne microorganisms entered the Iberian Peninsula from the west sector (northwest, west or southwest) during the period of study. However, full trajectories had different origins (e.g., European origin in the samples collected on 21 and 26 November, Atlantic origin for the one collected on 23 November or American origin for those collected on 22, 27 and 28 November).

During the first period, with cyclonic conditions, the trajectories flew mainly at low altitude. However, during the second period with anticyclonic conditions, the trajectories moved at high altitudes during most of the path and subsided near the Iberian Peninsula (Fig. 3). This subsidence is characteristic of the anticyclonic conditions observed during this period.

When the dry deposition model was applied, the distance from which microorganisms could reach the sampling point depended on their density and size. We calculated for each back-trajectory the farthest point from which particles with density of 1 g cm^{-3} and diameters of 1, 5, 10 or $20 \mu\text{m}$ could have come. Fig. 4 shows the distribution of the flying time for the four diameters and their comparison with the complete trajectories (i.e., without biological content). Dry deposition hardly had effect on the $< 1 \mu\text{m}$ spherical particles, so they could have the origin at any point in the complete trajectory. Particles with increased diameter could remain in the air for less hours, which places its possible origin in the western part of the Iberian Peninsula or the Atlantic Ocean. Therefore, particles larger than $20 \mu\text{m}$ can hardly come from other continental regions.

Fig. 5 shows the time of permanence in the air and back-trajectories (red) of four real non-spherical large microorganisms (length $> 20 \mu\text{m}$) with different ESD (9.8, 8.2, 7.9 and $6 \mu\text{m}$) captured in this study compared with the complete trajectory (black). It is observed that, considering the ESD, filamentous microorganisms with lengths $> 20 \mu\text{m}$ could remain in the air for more than 20 h. However, when the desiccation that these microorganisms could suffer during the fly is considered, the chance of remaining in the air increases much longer. This fact is illustrated in Fig. 6, where the dispersion capacities of a captured filamentous cyanobacterium with $92.1 \mu\text{m}$ length ($8.2 \mu\text{m}$ ESD) are represented for 25%, 50% and 85% of desiccation. The decrease in density makes the

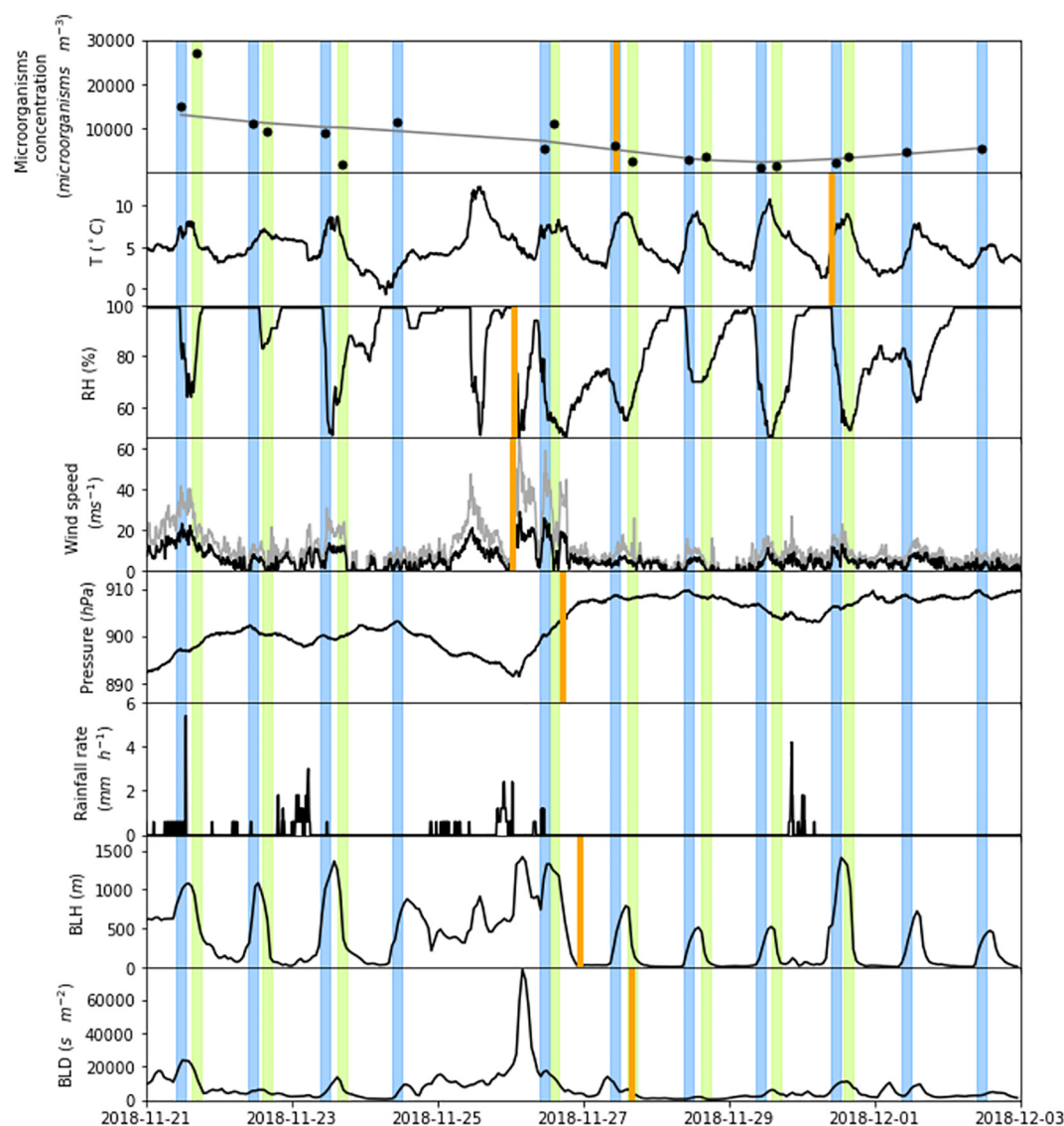


Fig. 1. Total airborne abundance recorded for each sample and time series of atmospheric variables sampling period. In the plot on the top, dots represent the “microorganism abundance” and grey line is the LOWESS non-parametric regression estimate. Temperature (T), relative humidity (RH), wind speed, pressure and rainfall rate were obtained from the nearest weather station (Colmenar Viejo) and has a temporal resolution of 10 min. Boundary layer height (BLH) and dissipation (BLD) were obtained from the closest grid point of the ERA5 reanalysis at hourly intervals. The blue and green columns indicate sampling periods in the morning and afternoon, respectively. The orange vertical lines show the change point for each variable listed in Table S5.

cyanobacterium to remain longer in the air. This allows estimating that, with sufficient desiccation, this microorganism would have had an intercontinental origin (North America) besides the probable local peninsular or nearby Atlantic marine origin. An extreme case of this can be seen in the dispersal capacity of the nearly 400 μm long (28.7 μm ESD) cyanobacterium found in this study. Its possible origin could be only local with a fully hydrated condition (density of 1 g cm^{-3}). However, with a dehydration of 85% (density of 0.15 g cm^{-3}), the organism could be transported ca. 450 km from the sampling station at the West coast of the Iberian Peninsula (Fig. S2).

4. Discussion

The dispersion of microorganisms through the air constitutes a subject of great interest to the scientific community. Although relevant progress has been done recently, many critical aspects remain to be uncovered. Most published biological studies about airborne microorganisms are focused on the biodiversity using Next Generation Sequencing (NGS) or culture driven experimental designs. Some of

them consider back-trajectories, but only few try to explain the interactions between the local atmospheric conditions and the abundance of airborne microorganisms. Moreover, to our knowledge, no study to date has suggested the origin of the microorganisms considering their morphological diversity and desiccation capability.

In this study we found similar range of microorganisms (10^3 – 10^4 microorganisms m^{-3}) than other published works on airborne particles over terrestrial environments (Harrison et al., 2005; Bauer et al., 2002). Like these works, we used traditional epifluorescence microscopy techniques to avoid the bias produced by the culture-dependent methods. Most of the airborne cells that we found were small, as in other studies (Mayol et al., 2014, 2017; Bowers et al., 2013; DeLeón-Rodríguez et al., 2013), but to the best of our knowledge the presence of non-spherical large microorganisms, up to almost 400 μm in length, has not been reported so far.

It is assumed that variability of airborne microbial community abundance is based on sampling location or timing. The highest airborne bacterial abundances correspond to grassland, urban and cropland sites and are observed during summer days in morning and evening hours

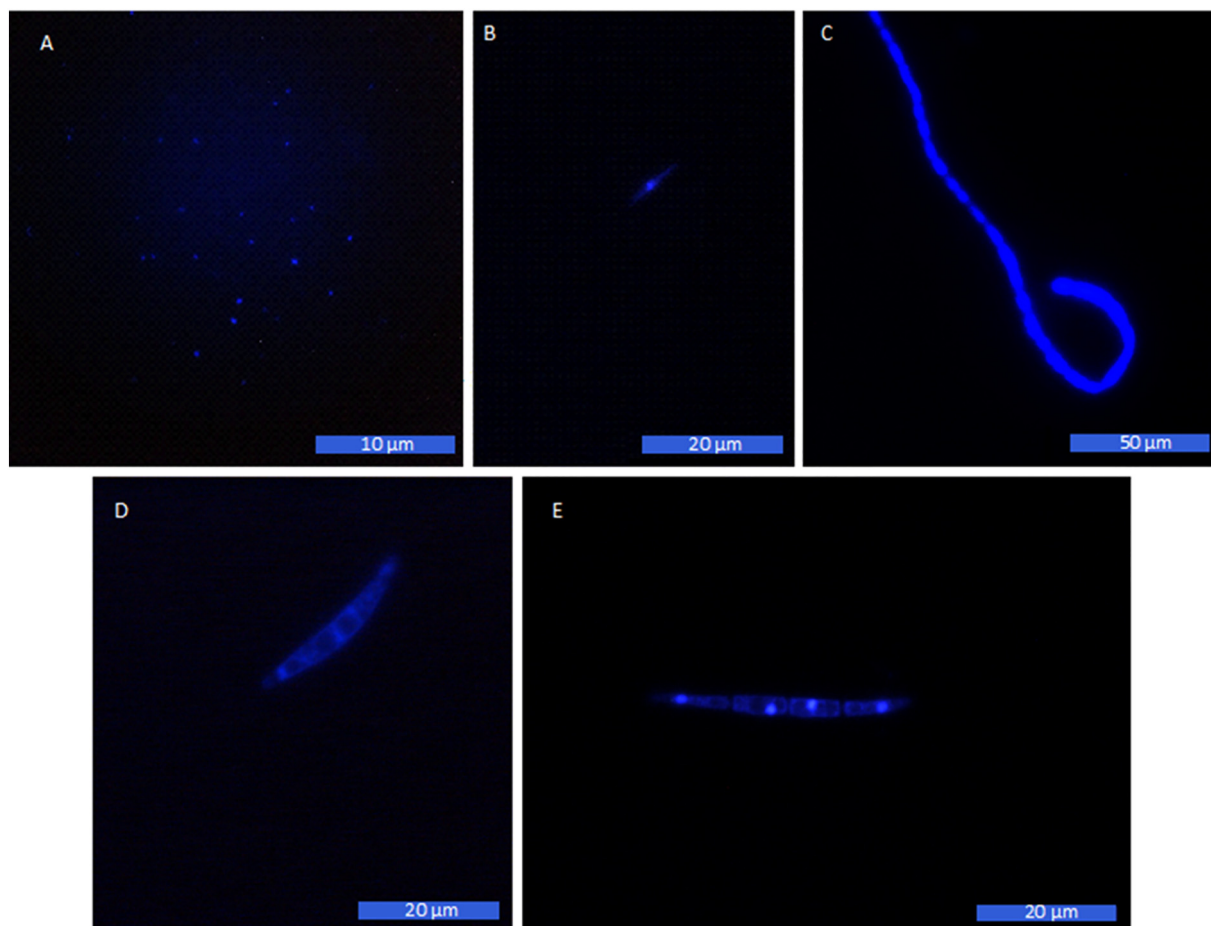


Fig. 2. Images of some airborne microorganisms found in the air samples. Measurement of the airborne microorganisms were carried out with an epifluorescence microscope (100 \times) with DAPI marker. (A) Non-defined prokaryotes and eukaryotes, (B) diatoms, (C) filamentous cyanobacterium, (D) desmidiaceae, (E) multicellular algae.

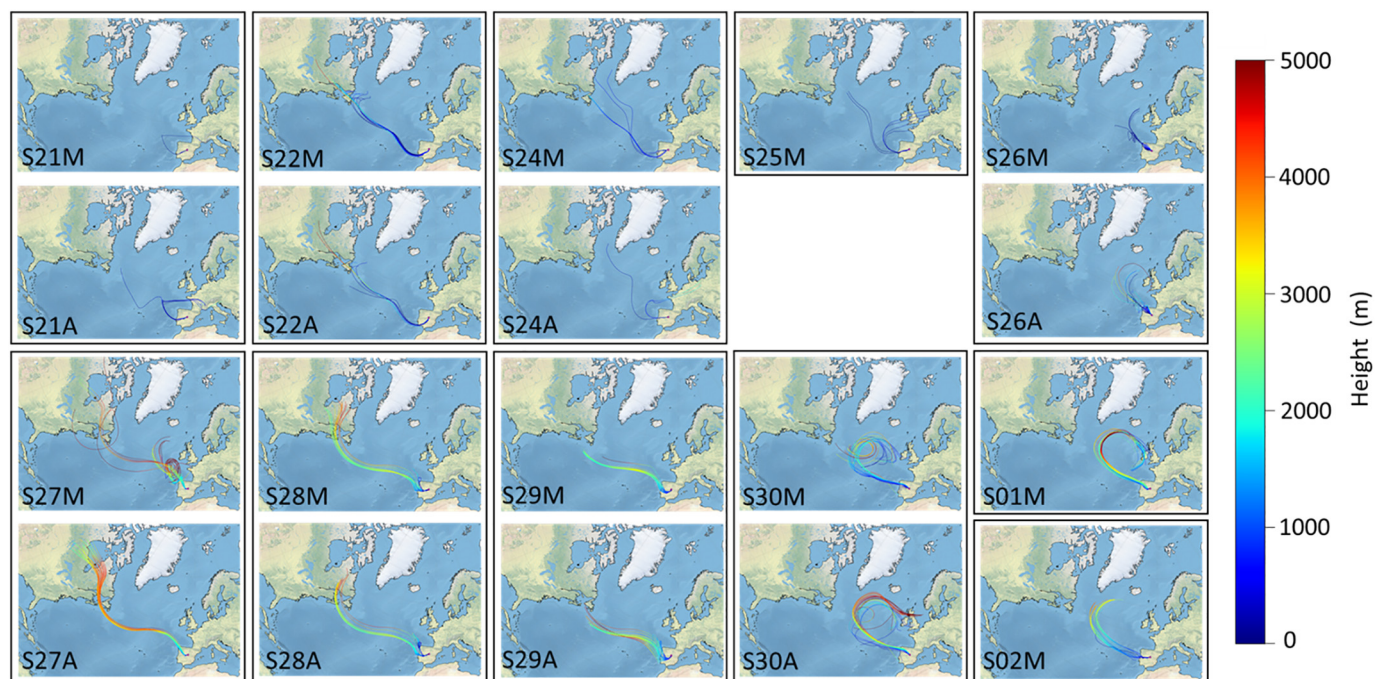


Fig. 3. For each sample, five-day HYSPLIT back-trajectories starting from the boundary layer of the sampling station each hour during sampling period. Colour scale indicates the height of the trajectory.

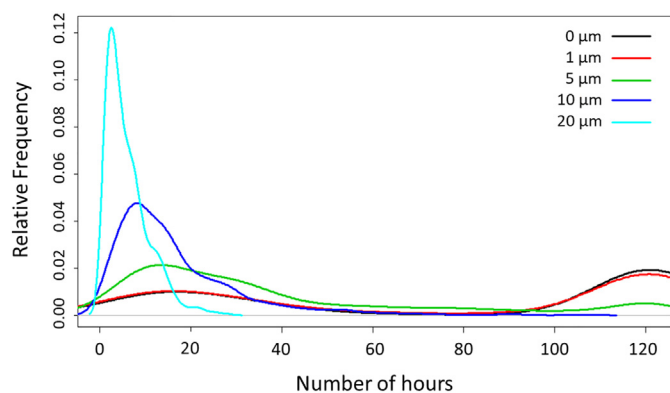


Fig. 4. Distribution of flying time (h) of trajectories from the boundary layer during the sampling period with dry deposition for spherical microorganisms with different diameters. Black line represents the trajectory without deposition.

(Tignat-Perrier et al., 2019; Bowers et al., 2012; Tong and Lighthart, 2000). Nonetheless, our study shows a dynamic behaviour of microorganism abundance with time and with significant evidence of a changepoint at the middle of the experimental period (27 November),

which corresponds with the variations of the synoptic and local atmospheric conditions. The first period was characterized by a higher concentration of microorganisms that decrease over time. The second one, presented a low load of microorganisms with a slight increase at the end of the sampling period.

The abundance of microorganisms in an air sample depends on two factors: (1) its initial airborne load (Rahav et al., 2019; Xu et al., 2019), and (2) air mass evolution, that is, the balance between deposition (impoverishing the load of microorganisms) and aerosolization (enriching it) (Rahav et al., 2019; Yuan et al., 2017). While the synoptic weather conditions drive where the air mass comes from, the balance between deposition and aerosolization might be driven by the atmospheric local features. Thus, the amount of airborne microorganisms captured at a precise sampling site can be explained, besides the original loading and the trajectory, by the local meteorological characteristics. Cyclonic events typically present increased wind speeds and frequent precipitation. While precipitation may scavenge the microorganisms of the air column (Yue et al., 2016; Tong and Lighthart, 2000), physical impact of the raindrops on the ground may contribute to the resuspension of the soil microorganisms (Joung et al., 2017; Tong and Lighthart, 2000). Wind speed also contributes to the aerosolization process (Burrows et al., 2009).

Our results suggest that during the cyclonic period, from 21 to 26 November, the aerosolization processes exceeded the deposition of

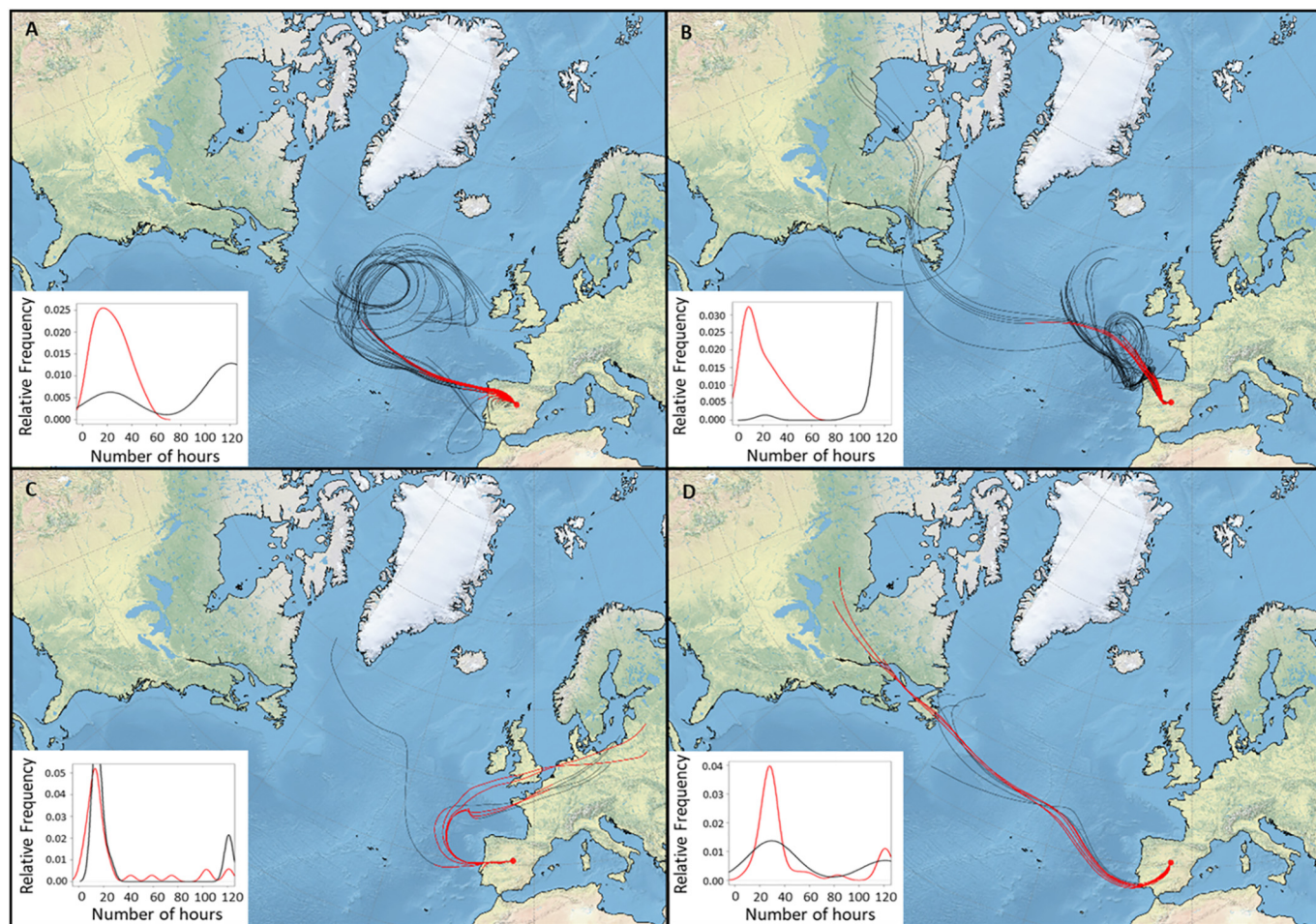


Fig. 5. Distribution of flying times (h) and five-day back-trajectories from the boundary layer during the sampling period for real large filamentous microorganisms (length > 20 μm) sampled in this study. Black lines show trajectories without dry deposition and red lines show trajectories with deposition considering the ESD of each microorganism. (A) Eukaryotic algae with len. 100 μm and diam. 2.5 μm (ESD = 9.8 μm); (B) cyanobacteria with len. 92.1 μm and diam. 2 μm (ESD = 8.2 μm); (C) eukaryotic algae with len. 36.5 μm and diam. 3 μm (ESD = 7.9 μm); (D) cyanobacteria with len. 22.5 μm and diam. 2.5 μm (ESD = 6 μm).

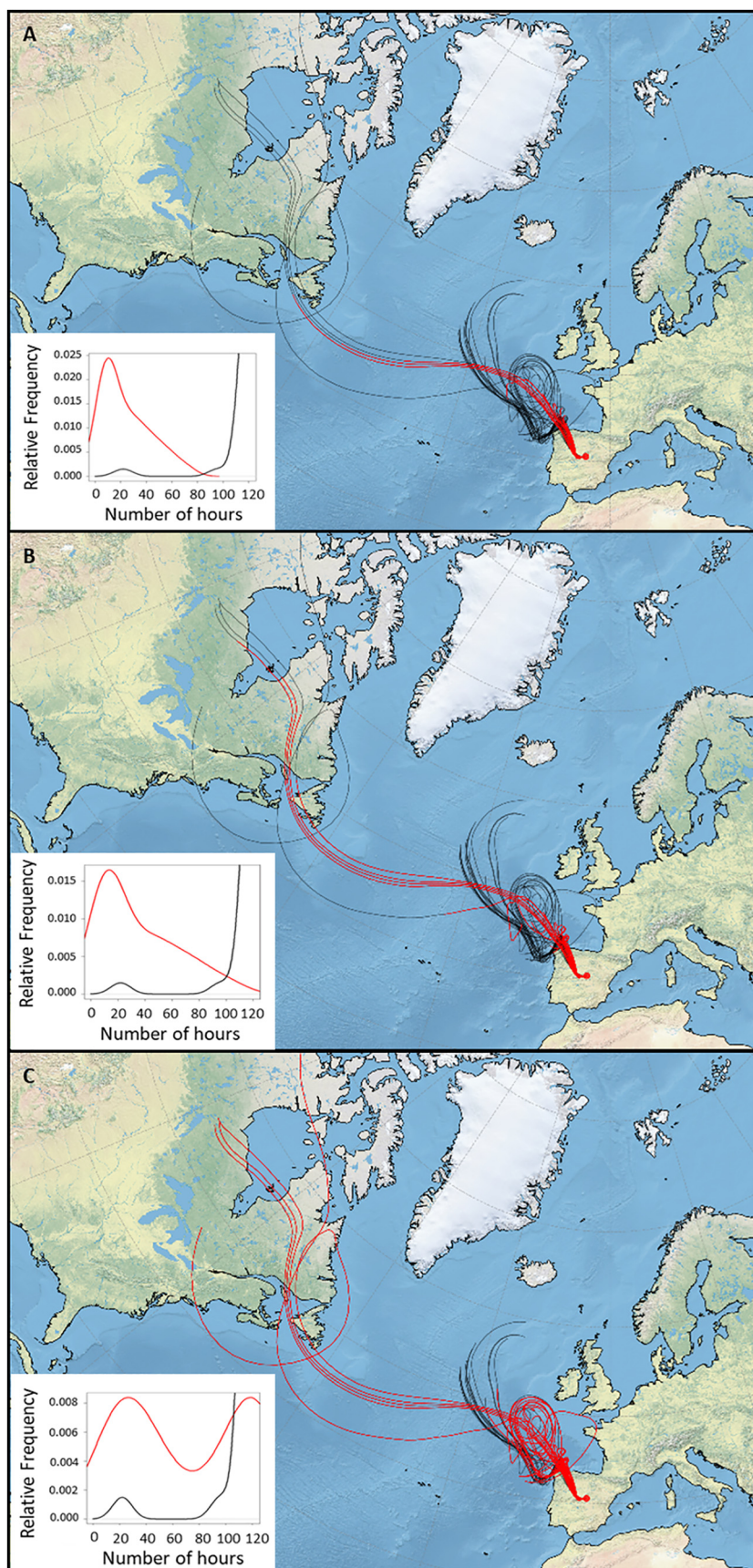


Fig. 6. Distribution of flying times (h) and five-day back-trajectories from the boundary layer during the sampling period of a real cyanobacterium sampled with len. $92.1 \mu\text{m}$ and diam. $2 \mu\text{m}$ (ESD $8.2 \mu\text{m}$) for different cases of desiccation. Black lines show trajectories without dry deposition and red lines show trajectories with dry deposition considering the ESD of the cyanobacterium. (A) 25% desiccation (0.75 g cm^{-3} density); (B) 50% desiccation (0.5 g cm^{-3} density); (C) extreme desiccation (85% desiccation) (0.15 g cm^{-3} density).

airborne microorganisms producing larger concentrations compared with the anticyclonic period from 27 November to 2 December. The period with more microorganism abundance was the one with a highest variability. In this case, Lagrangian coherent structures that occur more frequently in cyclonic conditions, may have contributed to increase the variability (Garaboa-Paz et al., 2015; Tallapragada et al., 2011). The anticyclonic conditions during the second period produced a stable environment, with low wind speeds and lack of precipitation (except during the cross of a weak front between 29 and 30 November). This would lead to steady conditions in the balance between aerosolization and deposition, and hence, to less variability of microorganism abundance. The small rise in microbial abundance recorded during the last samples collection (30 November and 1 and 2 December) could be related to a change in the synoptic conditions. Not relevant differences in the abundance of microorganisms between both periods due to their possible origins and geographical routes could be observed. Even though in each period they are variable, many of them are shared between both periods.

Our results are in agreement with most studies establishing the small microorganisms (e.g., prokaryotes) as dominant in the airborne community (Mayol et al., 2014, 2017; Bowers et al., 2013; DeLeón-Rodríguez et al., 2013). In fact, our samples show a remarkable constant size proportion during our sampling period. This dominance might be a consequence of the size spectrum of terrestrial biosphere: smaller microorganisms are much more abundant in all ecosystem's planet (Bonner, 2006). Likewise, their physical characteristics make them more likely to aerosolization and prone to remain longer in suspension (Wilkinson et al., 2012; Lara et al., 2011; Wilkinson, 2001). Even though the smaller microorganisms are easier to be dispersed and transported long distance than bigger ones, we have observed the presence of long filamentous microorganisms, highlighting the presence of really large microorganisms such as cyanobacteria up to almost 400 μm in length. Previous models in the literature typically considered the biological particles (pollen grains and spores) and dust, as spheres of unit density for simplicity (Monteith and Unsworth, 2008). Models based on spherical particles like Wilkinson's (Wilkinson et al., 2012) suggest similar results to those we have obtained. Microorganisms with diameter in the range of 1–5 μm show a remarkable successful aerial dispersal. For 20 μm diameter particles is very unlikely to be air dispersed (should deposit soon after aerosolization) and impossible for higher than 60 μm .

Wilkinson et al. (2012) proposed possible, but unusual, dispersal of big microorganisms on a large-scale by connections with unusual climatic events, such as dust storms, the migration of birds or air travel by human. However, most microorganisms are rod-like or filamentous and frequently their dimension exceeds 20 μm in length. In this contribution we show that when the size of these real long microorganisms is transformed into equivalent size (ESD), the sizes of microorganisms rarely exceed 10 μm . The dry deposition model implemented with the ESD indicate that those long microorganisms can be transported over long distances. This fact is favoured when realistic drying capacity in the models is also taken into account. When we incorporate ESD and potential dehydration in back-trajectories simulations, even quite long microorganisms can remain periods of more than 20 h in the air, arriving from quite distant locations, without ruling out that they may also have a closer origin. This is of great importance for ecosystems such hot deserts or polar regions (Antony et al., 2016; Crits-Christoph et al., 2016), where dehydration can be up to 98%. In this case, the dispersion of these non-spherical microorganisms is further increased by density reduction. The dispersion model that we show here allows to explain the possible long-distance dispersal of different genera of microorganisms recorded in various studies (Uetake et al., 2020; Archer et al., 2019; Mayol et al., 2017; Fahlgren et al., 2010). This provides an explanation of the appearance of microorganisms from ecosystems that do not exist in the vicinity of the sampling point, with individuals that are not evenly distributed throughout the planet (Brinkmeyer et al., 2003). The results presented here help to explain why dispersal

limitation is low in microorganisms and support the Baas-Becking (1934) assumption that 'everything is everywhere: but the environment selects', as well as a possible global-scale atmospheric dispersion of microorganisms (Griffin et al., 2017; Smith, 2013; DeWit and Bouvier, 2006; Baas-Becking, 1934).

5. Conclusion

In this study we explain the differences in abundance of the airborne organisms in relation to the changing meteorological conditions at local and large scale. Our main contribution to the study of this relationship is to consider atmospheric conditions as a whole and not as a series of meteorological independent variables. We also explore the origin of these microorganisms considering back-trajectories taking into account morphological and biological features as size and desiccation capability.

The results presented here provide a snapshot of a non-constant pattern of the abundance of the airborne microorganisms (ranging from 1.31×10^3 to 2.69×10^4 microorganisms m^{-3} of air) and its relationship with the local atmospheric conditions and characteristics of air masses that carry them. No one weather parameter alone explains the changes in microbial concentration, but it is found that the local weather conditions, as a whole, have a high influence on abundance. Despite the changes in microbial concentrations, the proportion of prokaryotes and eukaryotes are stable over time, with a dominance by small microorganisms between 1 and 5 μm . However, our results evidence the presence and the dispersal capacity of long microorganisms (>20 μm long), mainly cyanobacteria and eukaryotic algae with elongated shape, highlighting the presence of microorganisms with lengths up to 400 μm . Captures of microorganisms of the size of those we found in our experiment have been seldom reported. This is attributed to the fact that these organisms do not have the capacity for resuspension and dispersion due to their density. However, the incorporation of the equivalent size and the desiccation of non-spherical microorganisms into the dry deposition model to simulate the trajectories indicates that such organisms could come from locations hundreds of kilometres from where they were captured. Nonetheless, these results do not rule out the possible nearby origin. This provides an explanation of the appearance of microorganisms typical from ecosystems that do not exist in the vicinity of the sampling point. We believe it is necessary to continue with monitoring of airborne microorganisms around our planet from a multidisciplinary perspective, including biology, physics, meteorology and statistics. This would allow to understand better the limits of dispersal of microorganisms, their relationship with the atmosphere and their possible global-atmospheric dispersion of great importance for numerous fields of study.

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CRediT authorship contribution statement

Sofía Galbán: Methodology, Investigation, Formal analysis, Writing – original draft, Conceptualization, Writing – review & editing. **Ana Justel:** Methodology, Formal analysis, Funding acquisition, Conceptualization, Writing – review & editing. **Sergi González:** Methodology, Formal analysis, Conceptualization, Writing – review & editing. **Antonio Quesada:** Methodology, Funding acquisition, Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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