Microclimate, airborne particles, and microbiological monitoring protocol for conservation of rock-art caves: the case of the World-Heritage site La Garma Cave (Spain)

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Abstract

Cave heritage is often threatened by tourism or even scientific activities, which can lead to irreversible deterioration. We present a preventive conservation monitoring protocol to protect caves with rock art, focusing on La Garma Cave (Spain), a World Heritage Site with valuable archaeological materials and Palaeolithic paintings. This study assessed the suitability of the cave for tourist use through continuous microclimate and airborne particles monitoring, biofilm analysis, aerobiological monitoring and experimental visits. Our findings indicate several factors that make it inadvisable to adapt the cave for tourist use. Human presence and transit within the cave cause cumulative effects on the temperature of environmentally very stable and fragile sectors and significant resuspension of particles from the cave sediments. These environmental perturbations represent severe impacts as they affect the natural aerodynamic control of airborne particles and determine bacterial dispersal throughout the cave. This monitoring protocol provides part of the evidence to design strategies for sustainable cave management.

Keywords: Cave conservation, microclimate monitoring, aerobiology, airborne bacteria, biofilms, rock art

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1. Introduction

Caves often harbour significant geological and cultural features that, in certain cases, warrant their inscription on the UNESCO World Heritage List or under other legal national protection initiatives. Such recognitions can generate tourist interest and subsequently serve as a potential economic resource for regional and local authorities, as well as private entities. Preserving this valuable heritage requires the development of conservation strategies that aim to maintain and safeguard these spaces as closely as possible to their natural conditions. However, due to their stable and delicate nature, caves are highly vulnerable to disturbances arising from human activities, with tourism being a direct contributor (Baker and Genty, 1998, Saiz-Jimenez et al., 2011; Bontemps et al., 2022).

A first aspect to take into account is the spatio-temporal assessment of a cave in terms of the energy fluxes with the external environment controlling its environmental stability. Three energy levels can be found in a cave environment, from low to high passing through moderate energy (Heaton, 1986), considering some features such as the occurrence and periodicity of intense events (e.g. flooding), seasonal climatic events, or permanent streams of air current. When a particular cave or cave sector falls into the low-energy category, its microclimate is extremely stable, and often the highest energy event may be related to the seasonal recharge of drip water. In this case, the energetic equilibrium can be very easily unbalanced, especially in the deeper or more isolated sectors, where the stable climate can be easily disturbed (Constantin et al., 2021).

Before sound management policies can be drafted, implemented, and enforced, stakeholders must first have knowledge of the management requirements of each cave. This must be based on the inventory of cave heritage features to provide insights on its sensitivity and disturbance due to surface and subsurface anthropogenic impacts (Harley et al., 2011). Therefore, a fundamental and initial aspect of managing caves involves analysing the primary risk factors and implementing appropriate measures to prevent or mitigate them.

Cave tourism has a direct effect on the community of Bacteria and an indirect influence on Fungi and Archaea (Piano et al., 2023). The cave-dwelling microorganisms are, therefore, reliable and significant proxies for impacts on these underground environments and their use in management decisions should not be overlooked. Hence, microbiological monitoring, in conjunction with microclimate parameters monitoring (Fernandez-Cortes et al., 2006, Russell and Maclean, 2008; Garcia-Anton et al., 2014), must play a pivotal role in assessing impacts and formulating effective management decisions to protect subterranean sites.

Studies aimed at determining cave conservation status and preventing microbial outbreaks prioritise the identification and characterization of bacteria colonizing rocks, speleothems and sediments, as well as those present in the air and water (Mulec et al., 2017; Moldovan et al., 2020). This approach is equally crucial for controlling the biodeterioration of historical paintings and engravings (Martin-Sanchez et al., 2014; Saiz-Jimenez, 2015).

Acquiring knowledge about the environmental conditions of subterranean ecosystems prior to their tourist use is essential for distinguishing natural changes from those resulting from human activities (Sebela and Turk, 2014). The ultimate goal of environmental analysis is to decide whether there is a possible visit to the cave or not at all, and, if so, then to determine the sustainable visit regime (Hoyos et al., 1998; Lobo et al., 2013) and conditions that could prevent irreversible deterioration in the short and medium term.

This study introduces a microclimate and microbiological monitoring program specifically designed to promote the preventive conservation of rock-art caves. The study focuses on La Garma Cave, a renowned World Heritage site situated in Cantabria, Spain. Since its discovery in 1995, the cave has remained closed to the public and is exclusively accessible for archaeological research purposes. In a previous survey in 1998, shortly after its discovery in 1995 when only 64 people visited the cave with a total time of stay of 45 h and 30 min, it was stated that relatively few microbial biofilms were observed on the walls and sediments (Schabereiter-Gurtner et al., 2004). In order to assess the cave's suitability for tourist visits and ensure its conservation, we developed and implemented a tailored environmental study protocol, considering the cave as a protected ecosystem. This protocol encompasses the following components:

1. Continuous monitoring of the cave's microclimate at different points depending on its geomorphological characteristics: We conducted ongoing measurements to monitor and analyse the cave's temperature, relative humidity (RH), airflow, concentration of a tracer gas (CO_2), airborne particles and other relevant environmental factors. These data helped us gain insight into the processes governing the cave's natural microclimate patterns and any potential variations caused by external factors or human activities.
2. Study of the biofilms in the cave: We conducted an in-depth investigation of the biofilms that inhabit the rocks and sediments within the cave. By studying these microbial communities, we gained insights into their diversity, composition and ecology. This information was crucial for assessing the cave's overall ecological balance and understanding the potential impacts of tourist visits.
3. Monitoring of the cave air bacteria: This monitoring took place under various microclimate conditions, allowing us to evaluate any changes in the microbial population and their potential implications for visitor health and cave conservation.
4. Determination of induced environmental disturbances during controlled experimental visits simulating tourist activities.

By conducting this environmental study protocol, we aimed to gather scientific data and insights necessary for decisions regarding the cave's suitability for tourism. The findings will shed light on the needed balance between promoting responsible tourism and ensuring the long-term conservation of this natural cave ecosystem.

2. Study site

Spain and France are home to the majority of European Palaeolithic art, with their caves housing remarkable rock art paintings that are subject to special protection. Consequently, UNESCO has recognised the significance of these caves, leading to the declaration of Altamira Cave and the Palaeolithic cave art of Northern Spain as World Heritage Sites. In this context, La Garma Cave in Omoño, Cantabria, was included in the World Heritage List in 2008. Many caves in Northern Spain are open to visitors (Calaforra and Fernandez-Cortes, 2005), while others are being considered as potential show caves in the future.

La Garma Cave, discovered in November 1995 beneath a hill near the village of Omoño, forms part of a complex karst system comprised of galleries at various levels interconnected by vertical shafts. The cave opens on the southern slope of a hill at 187 m a.s.l. The cave is developed in Lower Cretaceous (Aptian-Albian) biomicritic limestones and the karstification process in the area was conditioned by the Cretaceous limestones fracturing system that led to a strong dissolution along sub-vertical fractures with a preferential NNE-SSW direction (Comas-Bru and McDermott 2015).

A warm temperate climate, fully humid with a temperate summer, prevails in the study area (AEMET-IM, 2011). This is classified as a “Cfb” type according to the Köppen-Geiger climate classification scheme (Kottek et al., 2006) and it is characterised by a coldest month ranging from -3 to 18 ºC, fully humid (no dry summer or dry winter), a temperature of the warmest month below 22 ºC and a minimum mean temperature of 10 ºC in at least four months. The mean annual temperature at the site during the monitoring and sampling period ranged from 13.92 to 13.98 ◦C, with the coldest months below 9 ºC in February and December, whereas the mean temperature of the warmest month was around 20.2 °C (July–August) (Sanchez-Moral et al., 2021). The mean annual total rainfall slightly exceeded 1000 mm and it was seasonally distributed with monthly maximums registered in February (240 mm/month, on average) and November (146 mm/month, on average). These averaged climate parameters are very similar to those reported by Rudzka-Phillips et al. (2013) for previous studied periods.

There are three easily accessible levels that have been the main objective of the study (Figure 1A):

- Upper Gallery: situated 80 meters above sea level, approximately, and 12-13 meters above the Middle Gallery. The cave entrance (named La Garma A) directly connects with the outside and serves as the current and single access point to the cave. This cave entrance faces SSW and leads into a small chamber (4x2 m), orientated along a SW-NE bearing (Peñalver et al., 2007). At the end of this chamber, a narrow passage 1.2 m wide connects with a second larger chamber (6.5x2.0 m), on the same bearing as the first. This leads into a third chamber of a similar size, and a narrow high-level passage that ends in a vertical shaft connected with the deeper levels of the subterranean system. This cave entrance is partially shaded since its surroundings are densely forested, mainly with *Quercus* spp and eucalyptus and dense undergrowth vegetation, over a soil whose thickness usually varies between 60 and 150 cm (Comas-Bru and McDermott 2015).”

- Middle Gallery: located 10-12 meters above the Lower Gallery. In the past, there was a direct connection to the outside through La Garma B, a small cave separated from the Middle Gallery by the growth of large stalagmites and a flowstone that currently blocks the passage. In recent years, several archaeological prospecting excavations have been conducted at this level. The Middle gallery is about 150 metres long, running from the hidden Garma-B entrance to its connection to the Lower gallery via a 30 m shaft. In the middle of this gallery, there is a connection with the Upper gallery through a descending ramp and a 7 m high shaft. Middle Gallery has narrow passages near the Garma-B entrance, measuring approximately 2x2 m with a circular section, which preserves the morphology of a phreatic tube associated with an ancient water spring, inactive since the progressive excavation of the karst system by the groundwater network. The dimensions of the gallery increase vertically towards the connection area of the Lower gallery, reaching a height of over 20 metres.
- The Lower Gallery constitutes the longest accessible section, situated 60 meters above sea level and is overlain by approximately 85-90 metres of limestone. This gallery constitutes a 300 m long horizontal passage, with narrow passages (2-3 meters, on average) with very variable heights, following the direction of the main fracture NNE-SSW, in the same way as the rest of the cave galleries. It consists of several sectors (I-IX) and previously had a direct connection with the outside, which is now blocked by detrital materials (Figure 1B) resulting from a massive movement of rocks, soil, and sediments down the slope around 16,000 years ago (Baldini et al., 2015). On the opposite end, Sector IX is connected by a shaft approximately 30 meters deep with the Bottom gallery in which is active a subterranean river and the water circulates and emerges outside through the "Fuente en Cueva" spring. The Lower Gallery harbours an exceptional Palaeolithic settlement, featuring paintings and engravings spanning from the early Upper Palaeolithic to the Magdalenian period (Ontañon, 2003; Arias and Ontañon, 2012). Its floor is extensively occupied by evidence of human activity, including animal bones, lithic and bone assemblages, mobile art, charcoal fragments, and more (Figure 1C). The captivating paintings and engravings within the cave depict various subjects such as bison, deers, horses, ibex, non-figurative strokes, and handprints (Figure 1D and 1E).

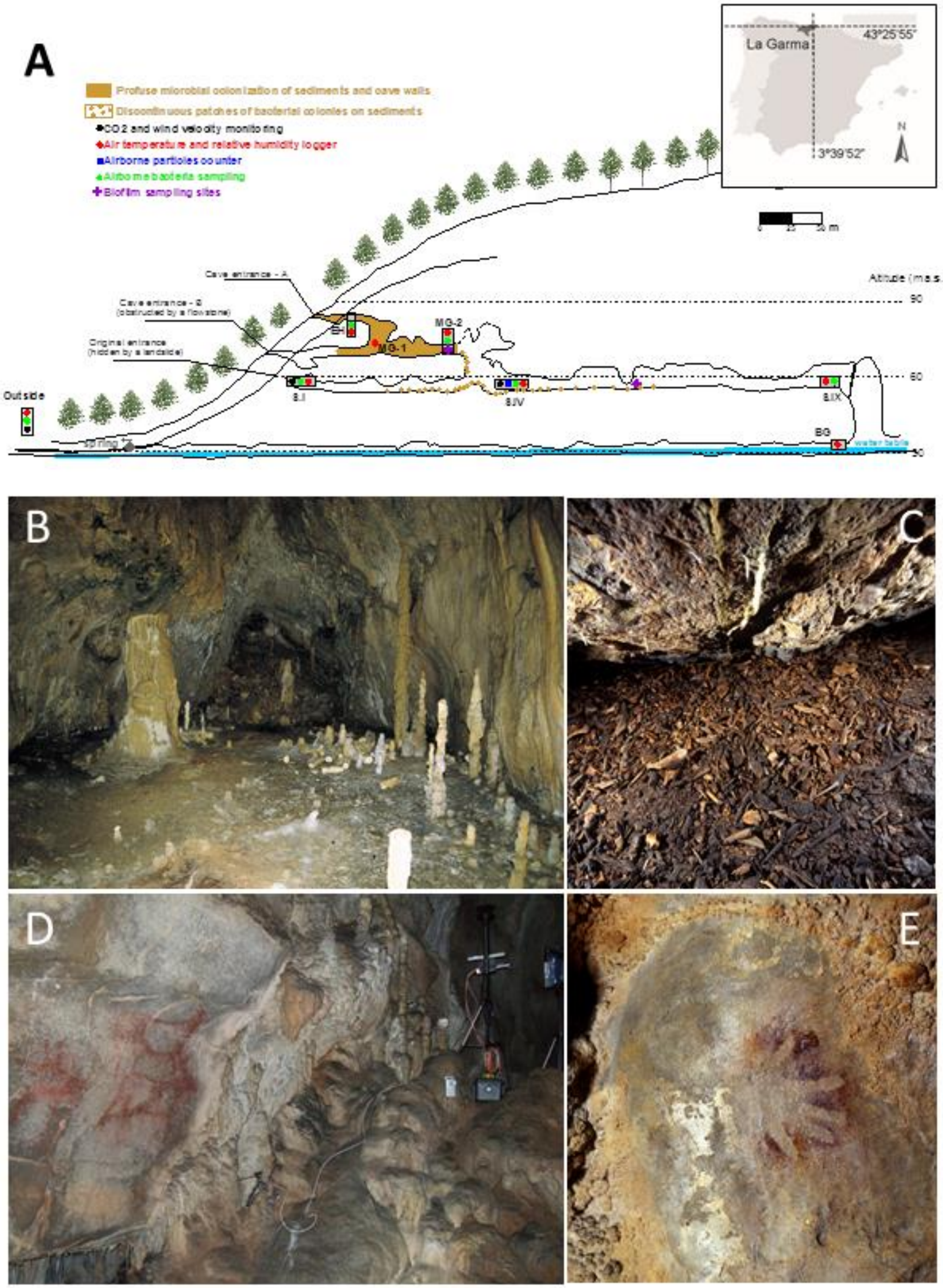
A
Profuse microbial colonization of sediments and cave walls
Discontinuous patches of bacterial colonies on sediments
CO2 and wind velocity monitoring
Air temperature and relative humidity logger
Airborne particles counter
Airborne bacteria sampling
Biofilm sampling sites
La Garma
43°25'55"
3°39'52"
N
0
25
50 m
Cave entrance - A
Cave entrance - B
(obstructed by a flowstone)
Original entrance
(hidden by a landslide)
Altitude (m.a.s.l)
90
60
30
EH
MG-1
MG-2
Outside
S.I
S.IV
S.IX
BG
spring
water table
B
C
D
E

Figure 1. (A): Network of microclimate monitoring stations and microbiological sampling points (airborne bacteria and biofilm over rock surfaces and sediments) distributed along the profile map of La Garma Cave. The main cave areas with microbial colonization are distinguished. EH, Entrance Hall; MG, Middle Gallery; S, Lower Gallery, labelled by sectors (I, IV and IX), and BG, Bottom gallery. (B): Dead end gallery in Sector I, blocked the connection with the exterior by detrital materials resulting from a breakdown around 16,000 years ago (Baldini et al., 2015). (C): Example of a Palaeolithic occupation area with the floor covered by animal bones, lithic and bone assemblages. (D) Environmental monitoring station near a rock panel with a composition of aurochs, ibex, horse and giant deer painted in red from the Early Upper Palaeolithic in Sector IV (station S.IV of Figure 1). (E) Negative hand stencil painted in red from the Early Upper Palaeolithic.

3. Material and methods

The monitoring protocol encompasses various components, including continuous microclimate monitoring, analysis of air quality and particulate matter, assessment of moisture levels, microbial analysis of cave sediments and biofilms, and mapping of rock-art conditions. These methods were carefully designed to provide comprehensive insights into the cave's environmental dynamics and potential risks to its preservation.

3.1. Monitoring of microclimate and cave air renewal

Cave microclimate conditions were monitored along different locations: one point in the Bottom gallery (near the underground river course), three points in the Lower Gallery (Sector I, IV and IX), one in the Middle Gallery and one in the Upper Gallery (Entrance Hall) (Figure 1A). Meteorological conditions outside the cave (temperature and relative humidity) were also monitored at the base of the hill where the cave is located. At the Middle and Upper galleries, Sector IX and outside the cave, the air temperature and relative humidity (RH) were recorded every half-hour with Tinytag TGP-4500 dataloggers (Gemini, West Sussex, UK). This device is equipped with a thermistor sensor (accuracy of ±0.5 °C between 0 and 40 °C and resolution: 0.01 °C) and an in-built capacitive sensor for RH (operating range between 0 % and 100 %, resolution of 0.01%, with an accuracy of ±2 % from 0 to 90 % and ±3 % from 90 to 100 %). The relative humidity of the cave air in Sectors I and IV was measured using a HUMICAP®180R probe (Vaisala, Vantaa, Finland), connected to a CR1000 logger (Campbell Scientific, Logan, USA). This probe has an accuracy of ±1% from 0 to 90% and ±1.7% from 90 to 100%, within a temperature range of 15 to 25 °C. Air temperature data from Sectors I and IV were obtained using a temperature logger with an external thermistor (SBE 56, Sea-Bird Electronics, Washington, USA), with excellent performance in terms of measurement accuracy (±0.002 °C, from -5 to +35 °C) and resolution (0.0001 °C). A spatially resolved monitoring of air temperatura was conducted seasonally using a handle thermometer (5611 T, Hart Scientific), in a pre-established network of points covering the Upper, Middle, and Lower Galleries.

CO_2 mainly fluctuates in well-ventilated caves by the internal circulation of air masses and depending on the air renewal and gaseous dilution by mixing with the local atmosphere at the exterior. The globally averaged marine surface annual mean was ~401 ppm during the monitoring period, accordingly to National Oceanic and Atmospheric Administration

(Lan et al., 2023). Therefore, this gas is an excellent tracer for air circulation and a trusted indicator of cave ventilation rate, both on a temporal and spatial scale. For this purpose, CO_2 concentration was hourly registered at sectors I and IV by using NDIR GMM222 transmitters (Vaisala, Vantaa, Finland), with a measuring range of 0–5000 ppm and an accuracy of ± 1.5% of full scale and ± 2% of reading. Each CO_2 transmitter was connected to a CR1000 logger (Campbell Scientific, Logan, USA). Grab CO_2 data was also periodically registered in the Sector IX, at the end of the main gallery near the shaft connecting with the underground river (Figure 1A), by air sampling and the subsequent analysis within 48 h of sampling for CO_2 concentration by a laser-based analyser (Picarro G2201-i, Santa Clara, USA). Air samples were collected with micro-diaphragm gas pumps (KNF Neuberger, Freiburg, Germany) and stored in 1-L RITTER bags. The same procedure was conducted in Sector I and IV aiming to validate the records by the NDIR probes.

The monitoring of the cave air dynamic was complementary with the continuous recording of wind velocity. Two-dimensional WindSonic ultrasonic anemometers (Gill Instruments, Lymington, UK) that measures wind speed and direction were installed at two key locations in the cave; sectors I and IV. Wind speed was measured in a range from 0 to 60 m/s, with a minimum detectable speed of 0.01 m/s, an accuracy ±2% of measurement and resolution of 0.01 m/s. Wind direction (range: 0° to 360°) was measured with an accuracy of ±3° and a resolution of 1°. Data were transmitted via SDI-12 protocol to each CR1000 logger. Additional data of daily rainfall and prevailing winds outside cave (wind speed and direction) was obtained from a station of the Meteoclimatic network (http://www.meteovillaverdepontones.es/meteodata/, model Davis Vantage Pro2 Plus) located at Villaverde de Pontones (43° 24' 48''N, 3° 42' 05''W), less than 3 km from the cave entrance.

Suspended particles (aerosols) become entrained and transported in the cave atmosphere; they are therefore a useful tool for delimiting in time the air mass exchange with the outside by advective forces and the potential biomass transport within the cave environment. For this purpose, a portable TSI Aerotrak Model 9306 (Aachen, Germany) airborne particle counter was used for the temporal monitoring of aerosol concentrations in Sector IV. This device works with a 2.83 L/min flow rate and counts up to bin sizes from 0.3 to 25 µm with ± 5% accuracy, logging up to 6 particle sizes simultaneously. Airborne particles were continuously registered over a year but, for this investigation, the particle analysis was focused on the most intense period in terms of air exchange with the exterior, i.e. during the late summer and early autumn.

3.2. Aerobiological sampling

Three sampling campaigns were carried out during 2015. In each sampling, a total of five points inside the cave and one outside were measured. The sampled points were (from the entrance to the end): Upper Gallery (Entrance Hall), Middle Gallery, and Lower Gallery (Sector I, Sector IV, and Sector IX). A sample from the exterior air was taken as control (Figure 1A). These periodic samplings were coincident with the main periods of cave air dynamic: ventilation (March 24, 2015) stagnation (November 19, 2015), and ventilation pulses stage at the end of summer (September 17, 2015), as reported by Sanchez-Moral et al., 2021).

The sampling was carried out with the Duo SAS (Surface Air System) Super 360 system. This equipment is a type of suction impact collector, which has been widely used in aerobiological studies. It allows the detection of a great diversity of cultivable microorganisms by filtering a preselected volume of air through two heads provided with a series of holes. Samples were taken in duplicate. The volume of filtered air was 100 liters. These 100 liters were set because at higher volumes the number of bacteria was too high for counting (Porca et al., 2011). The culture medium used to promote the growth of bacteria was trypticase-soya-agar (TSA, BD) with cycloheximide (Applichem, Darmstad, Germany) (50 µg / mL).

Viable counts and isolations in pure culture were independently performed for each colony type with different morphological characters, and for each replicate of air sample. The bacteria were isolated in TSA culture medium.

The final amounts of bacteria in each air sample were expressed as colony-forming units per cubic meter following manufacturer's instructions. The number of colonies counted on the surface of the culture plates was corrected for the statistical possibility of multiples particles passing through the same hole.

3.3. Sampling of microbial biofilms on speleothems and sediments

In order to compare cultivable bacteria in air and biofilms, five samples were collected from two cave sectors, Middle Gallery, and end of Sector IV. The samples were collected from sediments as grey biofilms (GC18R, GC19S, GC27A), and from speleothems as pink (GC20T) and yellow (GC21U) biofilms (Figure 2).

For the identification of isolated bacterial strains, amplification was carried out by means of the polymerase chain reaction (PCR) of the 16S ribosomal RNA (rRNA). Each PCR reaction contained 5 µL of 10X BioTaq buffer (Bioline, Randolph, Massachusetts, USA), 1.5 µL of 50 mM $MgCl_2$, 5 µL of a 2 mM deoxyribonucleotide (dNTP) mix (Invitrogen, Carlsbad, California, USA), 0.5 µL of each of the primers, 50 µM (Invitrogen), 0.25 µL of BioTaq DNA polymerase (Bioline) and 10-20 ng of template DNA. The volume of the reaction mixture was made up to a final volume of 50 µL with sterile nucleic acid- and nuclease-free water (Sigma-Aldrich). The amplification reaction was performed in a FlexCycler thermal cycler (Analytik Jena, Jena, Germany).

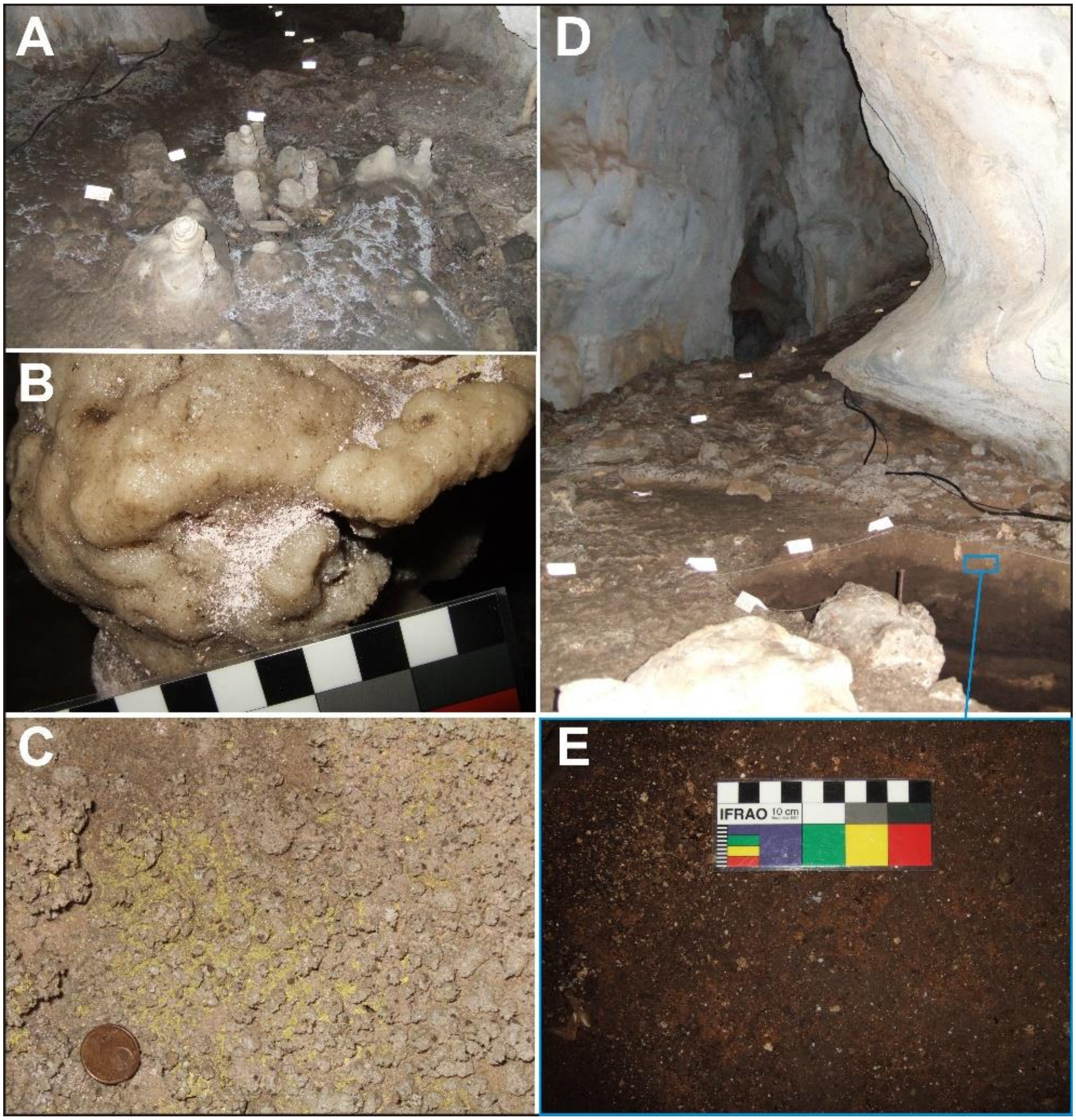

Figure 2. Biofilms on sediments, rock surfaces and speleothems in the Middle Gallery of La Garma Cave. (A) Grey and white biofilms patching a downward flowstone near the transit path (marked by reflective signs). (B) White and pinkish biofilms on a stalagmite. (C) Yellow biofilms on the rock surface. (D) general view of the archaeological excavation conducted in 1996, and E: detailed view of white biofilms growing on fresh sediments after excavation.

For 16S rRNA amplification, primers 616F (AGA GTT TGA TYM TGG CTC AG) and 1510R (GGC TAC CTT GTT ACG ACT T) (Juretschko et al., 1998; Echigo et al., 2005) were used. The program used for PCR consists of a first denaturation cycle at 94 °C for 2 minutes, followed by 35 cycles comprising 20 seconds of denaturation at 94 °C, 20 seconds of oligonucleotide annealing at 55 °C and 2 minutes of elongation at 72 °C. Finally, an extension cycle takes place at 72 °C for 2 minutes.

Positive PCR products were sent to Macrogen Inc. (Amsterdam, The Netherlands) for purification and sequencing using the same primer set. In order to approximate the phylogenetic identification of strains, the received sequences were compared, using BLASTn algorithm, to the non-redundant databases of sequences deposited at the National Center for Biotechnology Information (NCBI). Accession numbers of the bacteria were ON055943-ON056020, ON056022-ON056094, ON056097-ON056156 and ON076997-ON077025.

4. Results

4.1. Seasonal patterns of microclimatic conditions and air renewal in the cave

A combined graphical analysis of temporal evolution of air temperature and CO_2 concentration in La Garma Cave (Figure 3) indicated the alternation of two distinguishable seasonal patterns of cave aerodynamics over the year (Cuezva et al. 2016; Sanchez-Moral et al., 2021). Supplementary Figure S1 shows a schematic of the two seasonal aerodynamic patterns in La Garma cave throughout the year and the main air exchange flows.

Firstly, the cave underwent an intense air exchange with the outside from late November 2015 to middle April 2016, namely as ventilation period in Figure 3 and Supplementary Figure S1. The air outside, colder and denser than the cave air, entered the cave by the upper galleries (Upper and Middle Galleries) and, consequently, the cave air mass was mainly dislodged along these galleries. This air renewal process entailed an aerodynamic connection with the outside atmosphere, especially affecting the Upper and Middle gallery and Sector IV of the Lower gallery (Supplementary Figure S1-A).

During the ventilation period, marked thermo-hygrometric oscillations occurred on a weekly and even daily basis, causing pronounced and generalised thermal drops that reach -3 ºC in both Upper and Middle galleries (Figure 3). The Lower gallery was also affected, so Sector IV registered the minimum temperatures during February and March (roughly 11 °C, on average). However, the more distal areas such as sectors IX and I remained relatively isolated from this air circulation regime, registering very mild thermal dips. Particularly, Sector I recorded a quasi-constant air temperature throughout the year (12.97±0.06 °C) as a result of its geomorphological situation far from the convective airframe, which provided it outstanding thermal stability and, in turn, a greater environmental fragility to any potential disruption. Supplementary Figure S2-A compares the thermal stability of the Lower Gallery with the rest of the upper galleries, by means of the spatial distribution of the standard deviation of air temperature over a year. This parameter was below ±1.0 ºC in most parts of the Lower Gallery (from Sector IV to Sector IX) and decreased below ±0.5 ºC in Sector I. This thermal stability of the Lower Gallery contrasts with the temperature variations registered in the Middle and Upper galleries, where the standard deviation was above 1 °C (Supplementary Figure S2-A).

The Bottom gallery was not aerodynamically disconnected from the rest of the upper galleries during the ventilation period. The thermal decrease caused by the entry of cold external air in winter (ventilation period) was also noticeable in Sector IX, with a decrease of up to 0.3 °C, while in the Bottom gallery, this decrease was attenuated to 0.1 °C (Supplementary Figure S2-B). The cave air at both locations remained constantly saturated with water vapour (100% relative humidity).

The ventilation process also provoked a remarkable gaseous dilution of the cave atmosphere (Figure 3). The monitoring stations sited at sectors I and IV underwent simultaneous drops on CO_2 concentration until reaching minimum levels (around 500 ppm), i.e. close to the local atmospheric background. Within this period, sudden increases in CO_2 from this base level (roughly +500 ppm) were also recorded from December to February, in response to coeval increases in outdoor temperature above the temperature range of the cave that provoked timely isolations and short-term CO_2 recharges of the cave atmosphere.

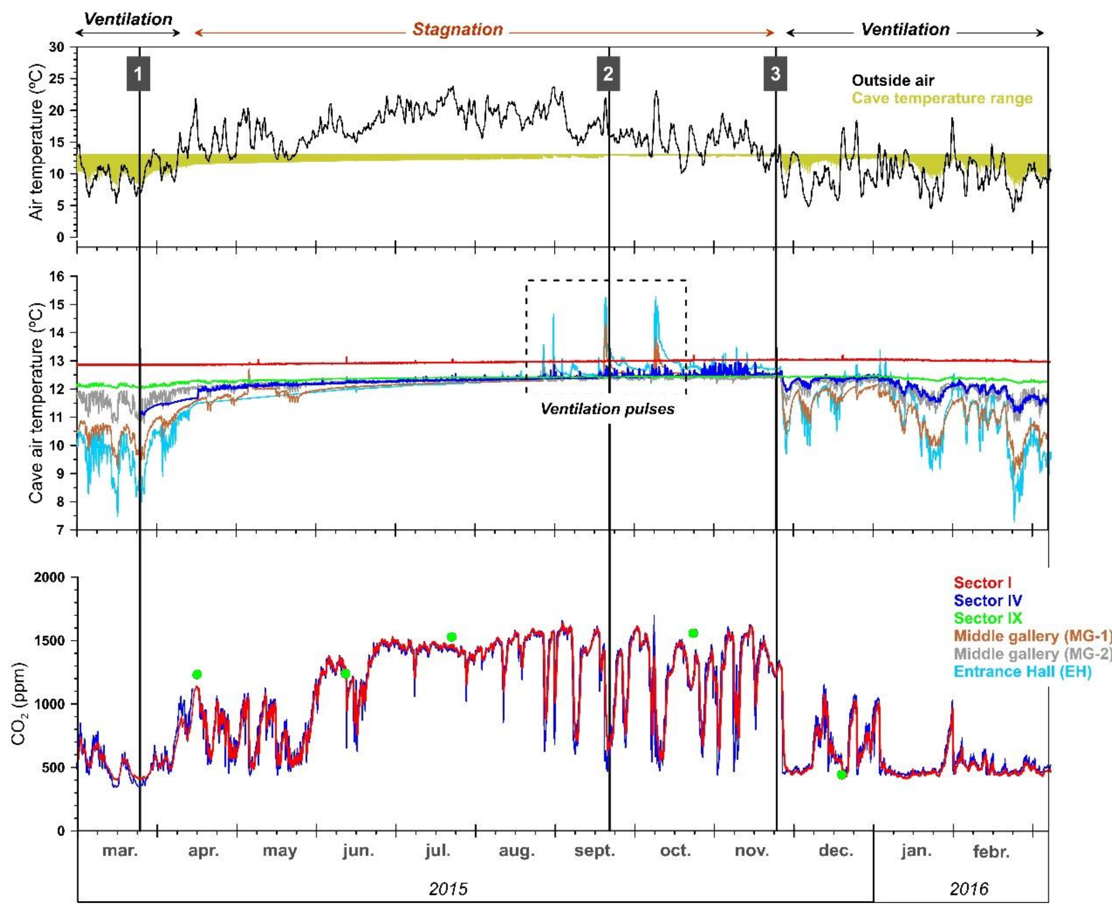

Figure 3. Time evolution of the air temperature at six monitoring points along the cave profile and comparison with the outside temperature and CO_2 concentrations at sectors I and IV. Campaigns of aerobiological sampling are labelled as [1], [2] and [3]. The locations of the monitoring stations are shown in Figure 1A. Ventilation pulses registered at the end of summer are analysed in detail in Figure 5.

Secondly, the stagnation period labelled in Figure 3 and Supplementary Figure S1, started once the outside air temperature increased progressively from mid-April to be clearly above the cave temperature range from the end of May onwards. Throughout the summer, the cave air temperatures tended to equalise, varying in a narrow range between 12.3 and 12.5 °C in all galleries, except for the Sector I that remained constant 0.5 °C above the rest of cave locations. CO_2 in sectors I and IV evolved together to a maximum value that was relatively stable at around 1500 ppm, between mid-June and mid-August. During summer, a negative temperature gradient between the exterior and cave environment was established and it remained almost constant. This provoked a vertical, air-density stratification and, consequently, the air exchange with the exterior by advective forces was blocked due to the main cave entrance (cave entrance A, Figure 1A) being located at a higher altitude than the rest of the galleries. This pattern of CO_2 and the spatial homogeneity of temperature indicate that a motionless cave air prevails, with minimum air exchange with the outside. During this period, the air circulation is limited to the upper galleries, with entry of warmer air, possibly through La Garma B entrance, and cooler air outlet through La Garma A entrance (Figure 1A). These episodic processes of air renewal cause short-term decreases in CO_2 levels from mid-June to mid-August (Figure 3).

Some outstanding pulses of intense ventilation at the end of summer and early autumn are noticed by a clear disruption of the thermal gradient between cave sites (labeled as "Ventilation pulses" in Figure 3). This intense ventilation is triggered once the wind regimen outside turns abruptly to prevailing warm winds with S-SW components and speeds higher than 10 km/h. Some short-period cycles of these warm southern are registered from late August to November. The disruptions in temperature match with sharp drops in CO_2 concentration in the cave air, which is quickly restored once these ventilation pulses cease. These ventilation pulses are extensively discussed below (subsection 5.1) in relation to the remarkable increase of airborne bacteria during this period.

The capacitive humidity sensors of the Tinytag TGP-4500 loggers were not accurate enough to record this parameter at near saturation conditions in water vapour. However, the records provided by the HUMICAP®180R probes, installed in sectors I and IV of the Lower gallery, did reflect the effect of the seasonal alternation of periods of isolation and ventilation of the cave atmosphere on the relative humidity. The average relative humidity in Sector I was 98.9 %, varying in a range between 98.4 and 99.2 %. This narrow range of humidity variation is a reflection of the high degree of insulation in this sector, although there were slight daily variations in humidity during the ventilation period (0.2%/day), which disappeared during the summer stagnation period. Relative humidity in Sector IV was subject to significant seasonal variations depending on the degree of cave ventilation and, therefore, on the greater or lesser renewal with cooler and drier external air. During the summer stagnation period, the relative humidity was very constant at around 95-96 %, with small daily variations always less than 1%/day. However, during the ventilation period, the average relative humidity dropped to 93%, with absolute minimums reaching 87-88 % and larger daily variations (1.8-1.9 %/day).

4.2. Cave aerobiology

Both, ventilation and stagnation periods are reflected in the aerobiological samplings, in addition to ventilation pulses at the end of the summer. Overall, some of the higher concentrations of bacteria in the cave air were obtained in September 2015, during the ventilation pulses period at the end of the summer.

In general, in the ventilation periods CFU m^{-3} values were lower in the galleries and sectors than in the stagnation period (Table 1). The unique exceptions were Sector IX in ventilation and Sector IV in ventilation pulses periods. Sector IV is a big open volume located after a shaft (Figure 1A) with air renewal process and relatively important concentration of airborne particles, such as it is reported in Figure 5. The CFU m^{-3} values in the Sector IX could be explained by the fact this sector is connected by a shaft with the Bottom gallery in which is active a subterranean river and the water circulates and emerges outside through the "*Fuente en Cueva*" spring.

Table 1. Bacterial CFU·m^{-3} in the air of galleries and sectors of La Garma Cave. Campaigns of aerobiological sampling are labelled as [1], [2] and [3] pointed in the Figure 3 and they correspond to March 2015, September 2015 and November 2015, respectively.

Air sampling location	Bacterial (CFU·m^{-3}) Ventilation [1]	Bacterial (CFU·m^{-3}) Ventilation pulses [2]	Bacterial (CFU·m^{-3}) Stagnation [3]
Upper Gallery (Entrance Hall)	N.D.	430	30
Middle Gallery	180	340	220
Sector IX	340	120	240
Sector IV	280	550	310
Sector I	190	220	400
Outdoor air	280	2580	490

N.D.: Not determined

Monitoring of the air temperature of the Bottom gallery with the subterranean river revealed greater short-term oscillations of air temperature in this area during the stagnation period (from July to October) compared to the rest of the year (Supplementary Figure S2-B). This oscillation may be related to the lower flow of the subterranean river during the low water level season, which favours the temporary air connection of the Bottom gallery with the exterior. The short-term thermal oscillations registered in the Bottom gallery were hardly transmitted to Sector IX, where the air temperature was kept almost constant during the summer.

A comparison of air temperature differences between the Bottom gallery and the end of the Lower gallery (Sector IX) also shed light on the aerodynamic connection between both locations (Supplementary Figure S2-B). The average temperature in the Bottom gallery was 12.61 °C, varying between 12.41 and 12.88 ° C over a year. The average temperature in the Lower gallery in Sector IX (S.IX) was 12.40 °C, varying in a range of 12.09 to 12.54 °C. Therefore, the temperature of the Bottom gallery is always slightly higher than the temperature of its connection to the lower gallery (station S.IX), with average differences ranging from 0.4 ° C during the ventilation period to 0.25 °C during the summer period when the cave is isolated (stagnation period). This thermal pattern allows the establishment of a convective cell that would favour an exchange of air masses between both locations because of differences in density, particularly during the ventilation period. This air mass exchange could explain some of the anomalous high concentrations of airborne bacteria, compared to neighbouring sectors of the Lower Gallery. During the summer isolation period, the temperatures of both zones tend to equalise, so this air exchange is attenuated.

The rates of bacteria inside the cave ranged from 30 CFU m^{-3} (stagnation, Entrance Hall) to 550 CFU m^{-3} (ventilation pulses, Sector IV) (Table 1). Outside, bacteria concentrations varied between 2580 CFU m^{-3} (ventilation pulses), and 280 CFU m^{-3} (ventilation). A comparison of the different sectors and galleries indicated that March, 2015 (ventilation) was the month with the lowest bacterial concentration in the cave (except for Sector IX). In September (ventilation pulses) and November (stagnation) the bacterial concentration was variable among the different sectors.

The bacterial composition of La Garma Cave air shows a relative homogeneity throughout the three samplings investigated and in the different galleries and sectors. In terms of both relative abundance and presence in each sampling across the cave, the presence of three species of *Streptomyces: S. avidinii*, *S. cyaneofuscatus* and *S. pratensis*, was remarkable. These bacteria were also found in the exterior of the cave, in varying quantities. Other abundant bacteria were *Peribacillus simplex, Micrococcus yunnanensis*, and *Streptomyces rhizosphaerihabitans*. Bacteria such as *Paeniglutamicibacter sulfureus*, *Pseudarthrobacter siccitolerans,* both formerly included in the genus *Arthrobacter* (Busse, 2016), *Arthrobacter* sp. (*A. oryzae/pascens*), and *Rhodococcus cerastii* were missing in the ventilation period.

Figure 4 shows the distribution and abundances of the top twenty most abundant bacteria in La Garma Cave during the periodic samplings. The abundant occurrence of *Streptomyces* spp., in the three sampling periods, is notable accompanied by *Pseudomonas helmanticensis* in the ventilation, *Paeniglutamicibacter sulfureus*, *Pseudarthrobacter siccitolerans* and *Arthrobacter* spp. in the ventilation pulses and *Oceanobacillus zhokaii* and *Peribacillus simplex* in the stagnation stages. The occurrence of these bacteria is common in most caves (Fernandez-Cortes et al., 2011; Garcia-Anton et al., 2014; Dominguez-Moñino et al., 2021).

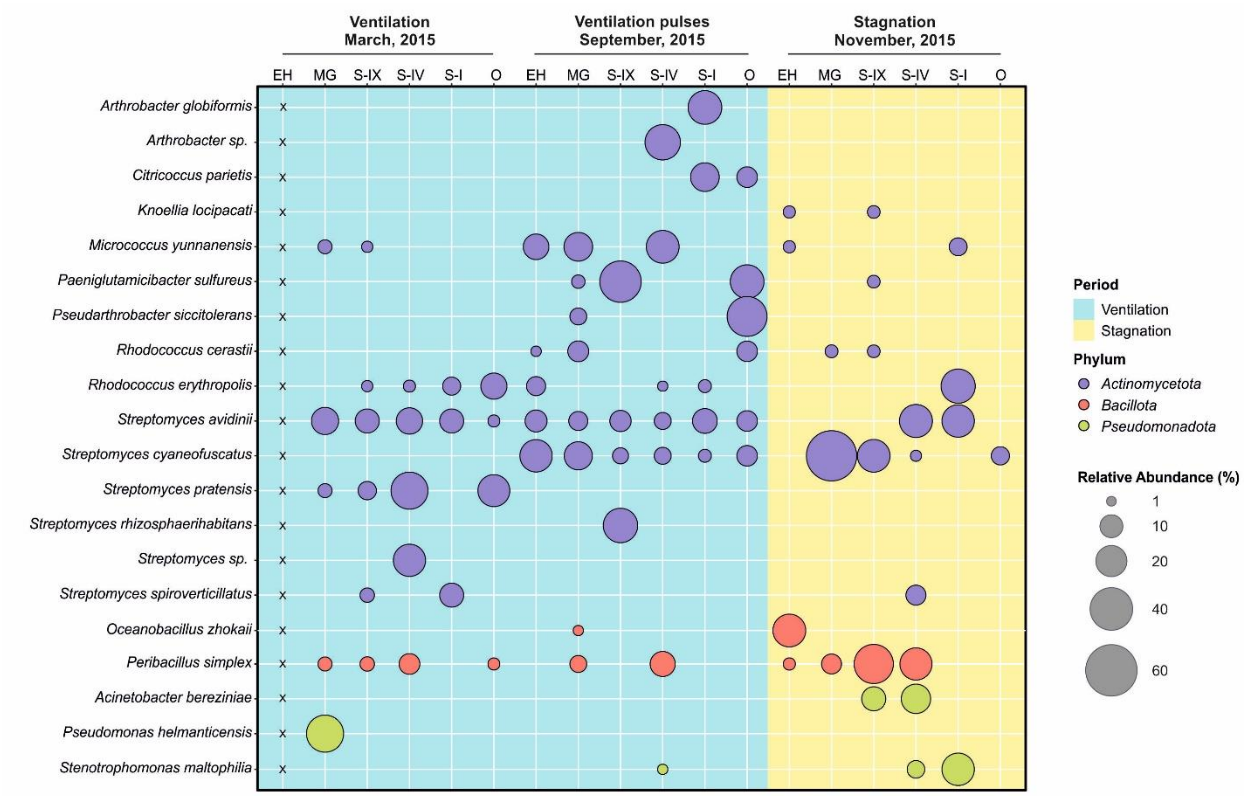

Figure 4. Bubble Plot. Most abundant bacteria, as distributed in each sampling point (O: Outdoor, EH: Entrance Hall, MG: Middle Gallery, S#: numbered sectors of Lower Gallery) and cave period (ventilation and stagnation). Missing values are represented by X.

It was remarkable the abundances of *Paeniglutamicibacter sulfureus*, *Pseudarthrobacter siccitolerans*, *Streptomyces* spp., *Arthrobacter* spp. and *Micrococcus yunnanensis* in the ventilation pulses period. In this period, drought conditions can be originated at the surface-air interfaces where bacteria are ubiquitous, particularly desiccation-tolerant *Arthrobacter* species (Stone et al., 2016) and spore-forming bacteria which are commonly found in wind transported dust particles (Azua-Bustos et al., 2019; Rao et al., 2020). However, in the stagnation period, *Streptomyces* and *Rhocodoccus* increased largely with respect to ventilation pulses period. *Knoellia locipacati* only appeared in the stagnation period, in addition to a few *Pseudomonadota* (*Acinetobacter bereziniae* and *Stenotrophomonas maltophilia*).

Supplementary Tables S1, S2 and S3 show the colony forming units of bacteria per cubic meter of air (CFU/m^3), their identifications and relative abundance.

Table 2. Bacteria identified in sediment and rock biofilms from La Garma Cave. The samples were collected from sediments as grey biofilms (GC18R, GC19S, GC27A), and from speleothems as pink (GC20T) and yellow (GC21U) biofilms, both of them along the Middle Gallery, and end of Sector IV.

Sample	Identification (% Similarity)	Accession Number
GC18R	*Microbacterium maritypicum* (99.83%)	ON076997
	Rhodococcus erythropolis (99.62%)	ON076998
	Priestia megaterium (99.76%)	ON076999
	Rossellomorea marisflavi (99.82%)	ON077000
	Peribacillus simplex (99.62%)	ON077001
	Pseudomonas chlororaphis (99.07%)	ON077002
	Lelliottia amnigena (99.89%)	ON077003
	Pseudomonas sp. (99.38%)	ON077004
	Pseudomonas sp. (99.81%)	ON077005
GC19S	*Streptomyces pratensis* (100%)	ON077006
	Priestia megaterium (99.79%)	ON077007
	Peribacillus simplex (99.90%)	ON077008
	Bacillus licheniformis (100%)	ON077009
	Cedecea lapagei (98.85%)	ON077010
GC20T	*Paenarthrobacter* sp. (98.59%)	ON077011
	Streptomyces cyaneofuscatus (99.86%)	ON077012
	Streptomyces avidinii (99.43%)	ON077013
	Peribacillus simplex (99.81%)	ON077026
	Bacillus licheniformis (99.60%)	ON077027
	Bacillus mycoides (99.63%)	ON077028
	Stenotrophomonas maltophilia (98.89%)	ON077015
	Stenotrophomonas chelatiphaga (99.89%)	ON077014
	Pseudomonas granadensis (99.30%)	ON077356
GC21U	*Metabacillus idriensis* (99.48%)	ON077016
	Peribacillus simplex (99.69%)	ON077018
	Paenibacillus amylolyticus (99.29%)	ON077017
	Cedecea lapagei (98.71%)	ON077019
CG27A	*Rossellomorea marisflavi* (98.53%)	ON077020
	Priestia megaterium (99.71%)	ON077021
	Peribacillus simplex (99.51%)	ON077022
	Stenotrophomonas maltophilia (98.89%)	ON077023
	Pseudomonas sp. (100%)	ON077024
	Enterobacter sp. (99.49%)	ON077025

4.3. Bacteria in biofilms from rocks and sediments

Table 2 shows that a few bacterial genera predominate in the isolates from biofilms collected on gray (samples 18R, 19S and 27A), pink (20T) and yellow (21U) biofilms: *Peribacillus simplex, Priestia megaterium, Pseudomonas* spp., *Streptomyces* spp., and *Stenotrophomonas* spp. Other less abundant genera *Cedecea*, *Microbacterium*, *Rossellomorea*, *Bacillus*, *Lelliottia*, *Rhodococcus, Paenarthrobacter, Paenibacillus, Metabacillus* and *Enterobacter* were also retrieved.

Among these isolates were included the four most frequent bacteria in the air of all the sectors and sampling campaigns: *Streptomyces cyaneofuscatus*, *Streptomyces pratensis*, *Streptomyces avidinii,* and *Peribacillus simplex*. Others species, also abundant in the air, such as *Bacillus licheniformis*, *Rhodococcus erythropolis* and *Stenotrophomonas maltophilia*, were represented among the isolates. A low abundant bacterium in the air: *Priestia megaterium* was present in the biofilms.

Likewise, a series of bacteria that were not found in the air were isolated from the biofilms, particularly members of the *Enterobacteriaceae* family, such as *Cedecea lapagei, Lelliottia amnigena* and *Enterobacter* sp. (*E. roggenkampii/asburiae*), which were present both in the isolates of the grey and yellow biofilms. This family includes coliform bacteria, which are part of the microbiota of the intestine of man and animals.

5. Discussion

5.1. Spatial stability of cave atmosphere and aerodynamic control of the airborne particles and bacterial dispersion

During early and mid-summer, the cave air renewal only affects the upper galleries through the inlet of warmer outside air into the cooler cave environment, triggering an outstanding condensation phenomenon at the beginning of the Middle Gallery and its connection with the Upper Gallery (see locations at Figure 1A). In general, effective condensation occurs when vapour pressure in the air is higher than on the rock surface (Fernandez-Cortes et al., 2013). The condensation of water vapour on the rock surfaces into the cave is triggered once the cave air temperature equalises with the dew point temperature under constant water saturation (relative humidity equal to 100 %) (Supplementary Figure S3), since these surfaces remain colder than cave air. Firstly, sudden rises of both temperatures are registered, inherent to the exothermic nature of condensation, and this is followed by a less abrupt thermal decrease once temperatures equalise. Condensation was particularly noted in these cave locations during our summer fieldwork campaigns. Condensation is naked-eye detected thanks to the presence of droplets covering the rock surfaces exposed to the main airflow paths and, particularly, on the vertical metallic structures and ladders installed in the shafts that allow access and connection between the three main levels of galleries, due to the different thermal properties. Condensation might favour the activation of microcorrosion processes of the rock supporting the paints or engravings, due to the CO_2, uptake by condensed water from the cave air and the subsequent calcite dissolution (Sanchez-Moral et al., 1999). In addition, condensation favors the biodeterioration processes linked to the metabolic activation of microorganisms present on the rock surfaces, or potentially colonizing them due to the presence of water (Martin-Pozas et al., 2023).

In these cave locations with high condensation rates, intense development of microbial colonization is also observed. An early colonization stage was already reported shortly after the discovery of La Garma Cave (Schabereiter-Gurtner et al., 2004). Based on the results from this initial study, it is noted that microbial colonization has progressed towards the internal zone of the Middle Gallery over the last two decades. On the contrary, thermal stability during most of the summer is a distinctive feature of the Lower Gallery, which is hardly affected by the ventilation phenomenon described for the upper galleries.

The situation of high thermal stability in the summer is frequently disturbed by short-period cycles of intense and warm southern winds, with speeds above 10 km/h. This change in the winds regimen activates a convective air cell in the upper galleries due to the inlet of warmer air through fissures and discontinuities likely located at higher altitudes than the Middle Gallery and its subsequent evacuation by La Garma A entrance. These ventilation pulses cause intense air temperature increases in the Upper Gallery and Middle Gallery of up to almost 3 °C, which reaches to reverse the previous thermal gradient between cave zones (Figure 5). Thus, the temperature of the Upper Gallery even reaches well above the temperature of the Lower Gallery (including Sector I).

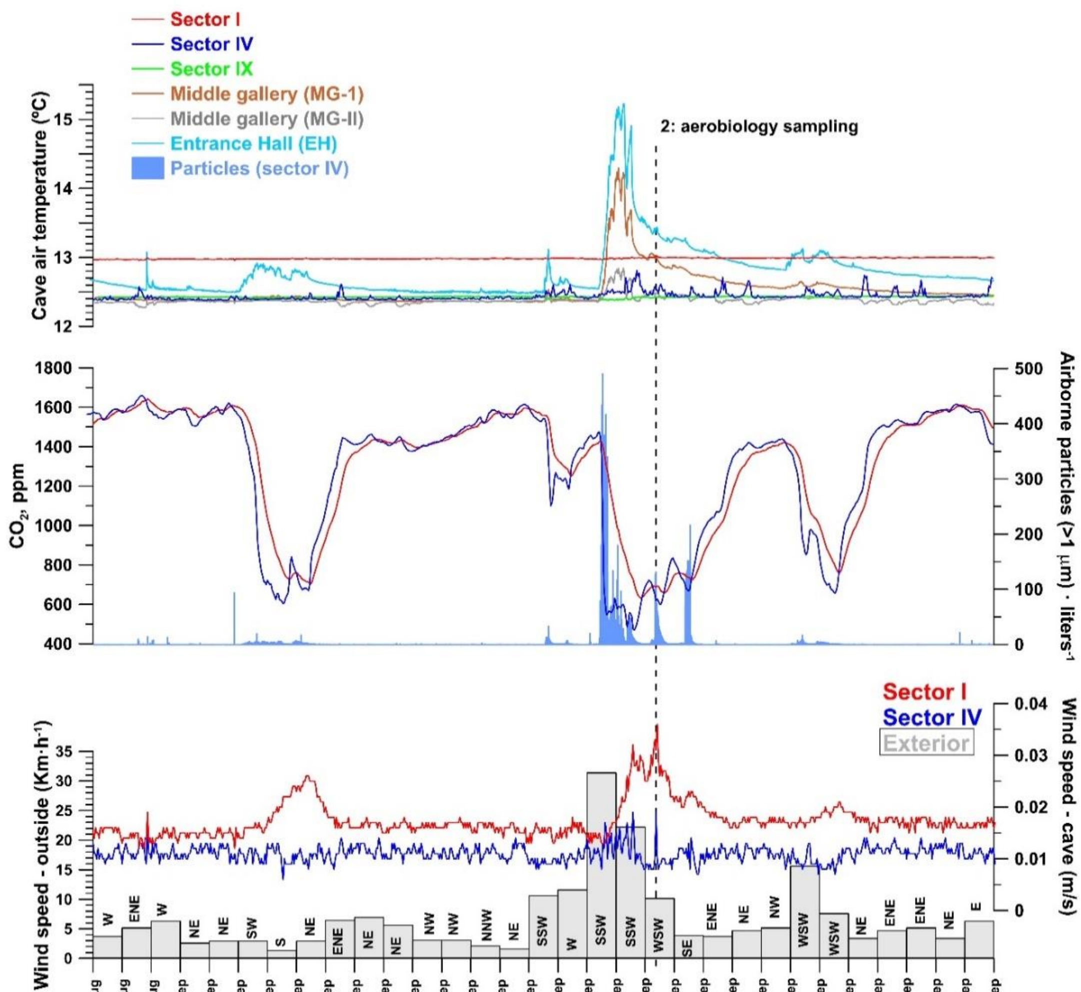

Figure 5. Detailed time-evolution of temperatures, CO_2 concentration, airborne particles and air speed inside La Garma Cave during the late summer. In the lower panel, the predominant speed and direction of the outside wind are indicated. The vertical dotted line labeled as 2 indicates the aerobiological sampling conducted on September 17th (see Figure 3), just after a marked ventilation pulse due to the incidence of warm and southern winds outside the cave.

Thermal instabilities between cave locations can last for several days and the resulting high ventilation provokes sudden and intense declines in CO_2 concentration throughout the Lower Gallery (Figure 5). The decreases in CO_2 levels reach roughly 800 ppm in Sector I and 1000 ppm in Sector IV. This generalised dilution of the cave atmosphere, therefore, affects both sectors simultaneously, which demonstrates the high intensity of the air renewal process throughout the entire cave environment.

The presence of coarse airborne particles in the cave environment would also corroborate the prevalence of intense ventilation. To check it, the records of 6 bin sizes of airborne particles, from 0.3 to 25 μm, were grouped into two classes with diameters higher or lower than 1 μm, aiming to simplify the comparative analysis with the rest of the cave environment parameters. The ventilation pulses by the southern wind also provoked the concentration of coarse airborne particles (> 1 μm) increasing above 100 particles/liter, which is distinctly from the almost null background levels recorded during previous periods of thermal stability and the absence of southerly winds (Figure 5).

The sudden increments of airborne particles are coeval to the higher motion of cave air. Once the convective cell into the cave environment is triggered by these southern winds, the cave-air speed also undergoes some remarkable increments of 1 to 2 cm/s in Sector I and some soft disruptions in Sector IV. The higher airspeed values registered in Sector I are due to a narrow blind-end gallery near the surface that provokes a tunnel effect on the cave air movement. However, sector IV represents a big open volume located in the base of a large shaft where air velocity changes are not easily detectable.

5.2 Microclimatic alterations by the presence of visitors

In order to establish the best conservation strategy, it is necessary to assess the vulnerability to the presence of visitors in the different areas of the cave. This is essential, especially in the event of a possible increase in the number of visitors or in case of adaptations for tourist visits. For this purpose, a detailed qualitative analysis was conducted to assess the impacts on microclimate parameters caused by occasional visits of research groups to the cave between April 2015 and July 2017 (22 days and 70 people).

Given the great variability of the research work carried out, the occasional visits had very variable characteristics, in terms of the number of members of the group, the route followed and the time spent inside the cave. For this reason, it was not possible to carry out an exhaustive quantitative and statistical analysis of the impacts generated. In the case of a possible opening to the public, it would be necessary to carry out a structured and rigorous experimentation, as well as its subsequent quantitative and statistical analysis, in order to evaluate the impacts and determine the viability of the proposed visitation regime.

The microclimatic disturbance analysis has been focused on the main monitored parameters (temperature, relative humidity, CO_2, wind speed, particles) in Sectors I and IV in the Lower Gallery. The effect produced by each entry of visitors was individually quantified, as well as the time required for the recovery of the values prior to the entry.

Based on the natural variation ranges of the microclimatic parameters in the different sectors and according to the previously determined seasonal ventilation patterns, the influence of the presence of visitors in the underground environment was evaluated.

The result of the analysis indicates that in Sector IV the presence of visitors for research activities caused slight or even undetectable effects on the main microclimatic parameters monitored. On air temperature, it caused slight increases with magnitude <0.1 °C equivalent to ≤0.02 °C per person. These values are lower than the average daily variation value during the monitored period, which ranges between 0.11 - 0.13 °C. The presence of visitors caused slight increases in the CO_2 concentration in the air, with maxima around 30 ppm, for an average daily CO_2 variation of 70 ppm. It also resulted in slight increases in relative humidity of 0.5%, compared to an average daily RH variation of 1.9%. No disturbances in wind speed were detected. Therefore, thermo-hygrometric impacts and perturbations on air CO_2 concentration, were of low magnitude and even undetectable in Sector IV; in all cases below the daily oscillation range and did not generate cumulative effects.

However, the effect of human transit on airborne particles is noteworthy in Sector IV because its pattern is different from that caused by natural dynamics, as in terms described for the outstanding pulses of intense ventilation due to southern wind at the exterior (Figure 5). Natural increases of airborne particles phenomena are also registered when the seasonal ventilation period is established, i.e. once the outside air temperature is permanently below the cave temperature range (Figure 6, from November 21st). The entry of outer air contributes with external suspended particles and also provokes its detachment and resuspension from cave surfaces. This leads to an overall increase in suspended particles, regardless of size. But, previously, in the absence of natural ventilation, human traffic even causes the most intense resuspension of coarser particles (>1 μm), reaching levels above 100 particles per litre of air, but with little effect on finer particles (Figure 6, from November 19th).

Ultimately, the analysis of the effects on the subterranean environment of the occasional visits for research in the cave has shown that in Sector IV they are moderate and in general of a lower range than those caused by natural environmental dynamics. This fact is related to the morphology of the Lower Gallery in this area, a big open volume located at the base of a large shaft, with a very narrow section and high ceilings that allow hot, humid and CO_2-enriched air generated by visitors' breathing to be directed towards the higher areas. Therefore, the impacts are not reflected in the areas closest to the floor and the painting panels, where the monitoring equipment is located (Figure 1D).

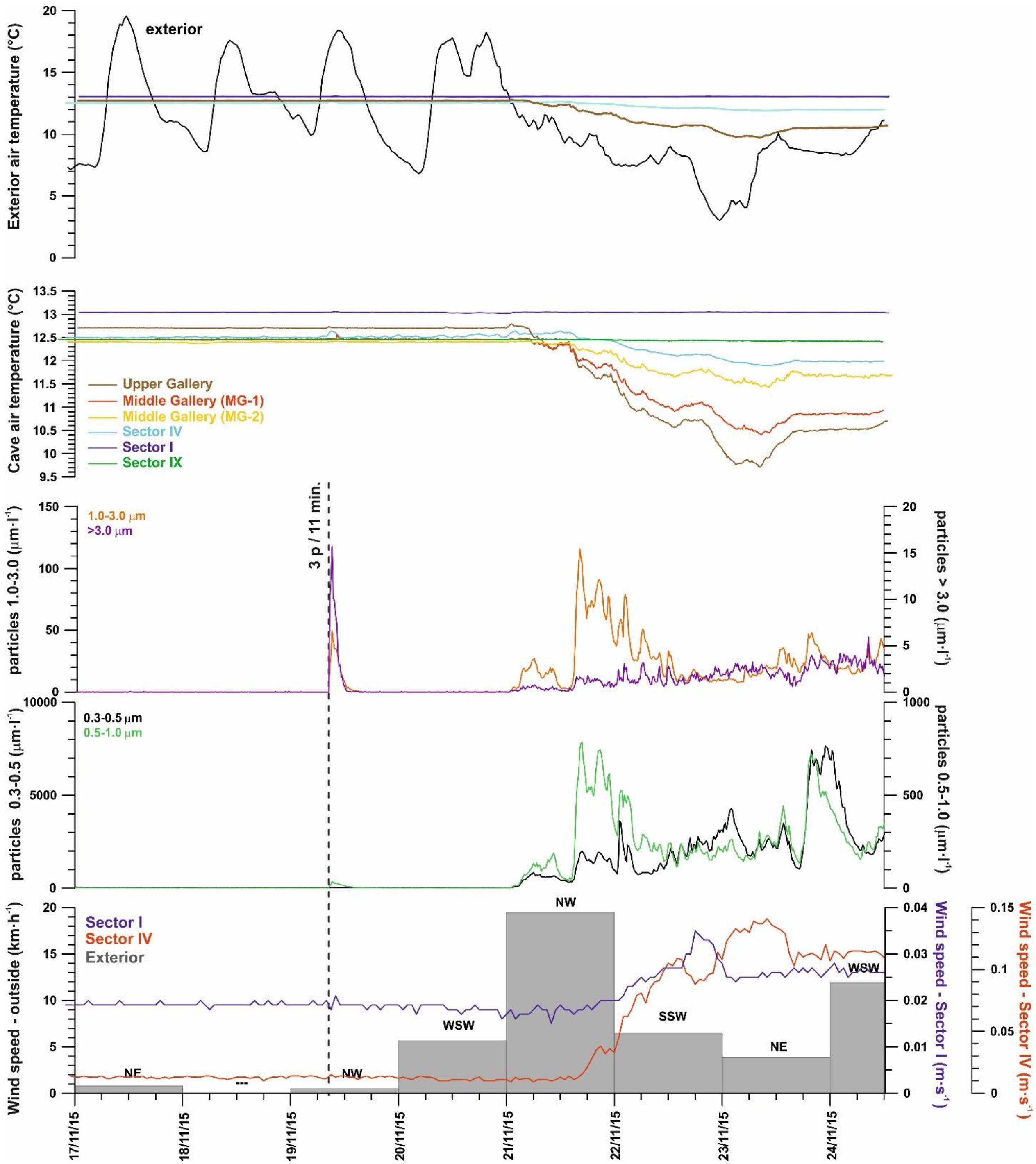

Figure 6. Example of the effect caused by the transit of people in Sector IV on suspended particles per liter of air, in relation to the thermal regime. On 19 November, the presence of 3 people for 11 minutes for the development of microbiological studies causes the resuspension of particles >1 µm with little lifting of small particles (≤1 µm). Two days later, from 21 November, with the change of aerodynamic regime, natural dynamics cause an increase in coarse particles of the same magnitude, but in this case also accompanied by resuspension of fine particles.

In Sector I, the presence of visitors for research activities generated impacts on air temperature very close to or above the daily oscillation range and mostly also above the monthly variation. Table 3 shows the quantification of the thermal impacts produced by 9 of the visitor's entries, selected given the homogeneity of the groups that allow the comparison of impacts according to the effect of the time spent and the entry of consecutive groups. This table summarises the duration of the disturbance, the recovery time and the relative impact per person for each group of visitors, compared with the daily and monthly variations of air temperature at Sector I under natural settings, i.e. without any entry of visitors.

Overall, entries with time spent in the Sector I of up to 30 minutes caused an average temperature increase of 0.08 °C, four times the average daily oscillation value (0.02 °C) and well below the total annual variation range of the monitored period (0.23 °C). The time interval required for the recovery of previous temperature values after the leaving of the groups of visitors generally ranged between 1 and 2 hours. In the case of very close entries, on the same day or on consecutive days, a very significant increase in these recovery times was detected. Thus, for example, there were two consecutive entries on the same day (on August 26th) with an interval of 23 minutes between them and the recovery time was extended to almost 4 hours (Table 3).

ENTRIES		IMPACTS				NATURAL PATTERN	
mm/dd	Persons / group	Disturbance Int (h:mm)	T Impact (ºC)	T Imp / Pers (ºC)	Recovery Int (h:mm)	∆T day (ºC)	∆T month (ºC)
06/01	3	0:16	0.05	0.02	1:30	0.02	0.07
08/26	4	0:09	0.05	0.01	> 00:23*	0.02	0.04
08/26	4	0:10	0.07	0.02	3:45	0.02	0.04
09/17	3	0:14	0.14	0.05	2:00	0.02	0.07
11/19	3	0:11	0.07	0.02	1:15	0.02	0.03
02/17	3	0:13	0.07	0.02	1:00	0.03	0.05
06/15	4	0:30	0.14	0.03	2:10	0.02	0.04
08/17	4	6:20	0.83	0.21	> 15:30*	0.01	0.05
08/18	3	7:30	0.46	0.15	8:00	0.01	0.05

Table 3. Natural temperature pattern and human-induced disturbance in Sector I of the Lower Gallery. Month/Day (mm/dd); Total number of persons per group [Persons/group]; Disturbance interval or time spent in Sector I [Disturbance int (h:mm)]; Increase in air temperature due to the presence of group of people in Sector I [T Impact (°C)], Relative thermic impact per person [T Imp / Pers (°C)]; Average time interval required for recovery of initial values [Recovery Int (h:mm)]; Monthly average of the daily oscillation of air temperature [∆T day (°C)]; Monthly average temperature oscillation [∆T month (°C)]. (* Indicates that the temperature is not recovered before the next entry of visitors, which implies a long-term cumulative effect).

A particularly example of strong disturbance occurred during a photographic work campaign, carried out over two consecutive days and with long duration stays (17 and 18 August 2016, 6:20 and 7:30 hours respectively). In this case, a mean thermal impact of 0.64 °C (0.83 and 0.46 °C) was recorded, which almost triples the total annual variation range (Supplementary Figure S4 and Table 3). In addition to this strong impact on air temperature, there was a clear cumulative effect, as the temperature did not recover the previous values of day 17 when the entry occurs on day 18, 15:30 hours later.

In this case, and given that the entrance to Sector I was for the purpose of carrying out photographic studies, we should also include other factors that we do not know about, such as the use of portable lighting systems, which causes the amplification of the thermal impacts both in quantity and duration. This conclusion is supported by the fact that the increase in air CO_2 concentration caused by the 4 visitors on the first day can be considered moderate-low (Δ 170 ppm) and that generated by the 3 visitors on the second day is practically null (Supplementary Figure S1). This together with a rather stable heating effect seems to indicate that the energy sources used during the photographic session were the main responsible for the environmental impact.

5.3. Bacteria communities of La Garma Cave and its contextualization concerning other subterranean environments

There are few airborne bacteria that were widely distributed in all the sampling periods and galleries of La Garma Cave, including *Streptomyces pratensis, Streptomyces cyaneofuscatus*, *Streptomyces avidinii*, *Peribacillus simplex, Micrococcus yunnanensis* and *Rhodococcus erythropolis*. This indicates that they were not dependent on air flows and could be considered as cave dwellers. In fact, four out of five (*Streptomyces pratensis, Streptomyces cyaneofuscatus*, *Streptomyces avidinii*, *Peribacillus simplex*, and *Rhodococcus erythropolis*) were also isolated from the biofilms paving the cave sediments. The abundance of these bacteria in the air of La Garma and other caves (Dominguez-Moñino et al., 2021) is in accordance with their spore-forming capacity, or ability to form elementary mycelia that fragments into short rods and cocci of most *Rhodococcus* (Jones and Goodfellow, 2012).

Species of the genus *Streptomyces* were practically present in all samplings and studies on caves. They were recorded in air, speleothems and sediments (Cuezva et al., 2012; Garcia-Anton et al., 2014; Martin-Sanchez et al., 2014; Belyagoubi et al., 2018; Gonzalez-Pimentel et al., 2020; Martin-Pozas et al., 2020; Miller et al., 2020). *Streptomyces avidinii* was previously reported from Spanish caves, such as Ardales (Fernandez-Cortes et al., 2011), Gruta de las Maravillas and Tesoro (Dominguez-Moñino et al., 2021), as well as Azorean volcanic caves (Riquelme et al., 2017).

Other widely distributed genus in caves is *Peribacillus,* formerly classified as *Bacillus* (Patel and Gupta, 2020). The genus *Bacillus* and their segregated genera were frequently isolated from caves (Fernandez-Cortes et al., 2011; Wang et al., 2012; Garcia-Anton et al., 2014; Dominguez-Moñino et al., 2018, 2021). *Peribacillus simplex* was relevant for their abundance and frequency in La Garma. This abundance is comparable to that of Ardales Cave (Dominguez-Moñino et al., 2021).

The genus *Micrococcus* is one of the most common among cultivable airborne bacteria. The abundance in caves may be due to its great versatility to colonise extreme environments and the ability to metabolise a wide range of substrates. This species was also found in other Spanish caves (Jurado et al., 2022) and it appears to be adapted to the relatively low cave temperature (12 °C). The abundance of *Micrococcus yunnanensis* in La Garma galleries and sectors was remarkable, and the fact that it was not found outside the cave would support the statement that the cave air is mostly populated by indigenous species characteristic for the subterranean environment, such as it was reported by Dominguez-Moñino et al. (2021).

The genus *Rhodococcus* is ubiquitous in soils and *R. erythropolis* and *R. cerastii* were the most abundant species in La Garma. *Rhodococcus* spp. are common in caves (Sanchez-Moral, 2014; De Mandal et al., 2015); in particular, *R. erythropolis* was very abundant in Castañar, Lascaux and Altamira caves (Porca-Belio, 2011; Martin-Sanchez et al., 2014; Sanchez-Moral, 2014).

An interesting feature from the point of view of the conservation of both the rock supporting the prehistoric paintings and the speleothems is that some airborne bacteria identified in La Garma Cave (Supplementary Tables S1, S2 and S3) can colonise cave mineral substrata. In this respect, the role of some bacteria in the precipitation of calcite is notable. Strains of the species *Stenotrophomonas maltophilia, Paeniglutamicibacter sulfureus, Pseudarthrobacter sulfonivorans, Sporosarcina aquimarina*, and *Peribacillus muralis* were reported to induce the formation of calcite and vaterite in the laboratory (Rusznyák et al., 2012; Otlewska et al., 2016; Enyedi et al., 2020; Saracho et al. 2020).

Regarding the bacteria isolated from biofilms, the occurrence of the *Enterobacteriaceae* family in caves was previously discussed in Altamira Cave (Laiz et al., 1999; Jurado et al., 2010, 2014). In this cave, it was found that this family of bacteria was present in the dripping waters and they entered the cave by infiltration from the top soil subjected to cattle management. The presence of *Lelliottia amnigena* (= *Enterobacter amnigenus*) in the waters of Altamira was interesting, in addition to several other species of *Pseudomonas* and *Bacillus* from polluted environments that were not found in La Garma Cave biofilms. The occurrence of members of the *Enterobacteriaceae* family is related to the presence of animals in the cave (Brenner and Farmer III, 2005; Köck et al., 2018). A survey on *Enterobacteriaceae* indicated that species related to those isolated from the biofilms were previously found in other caves. Thus, *Enterobacter asburiae* was isolated from bat guano (Tomova et al., 2013; Banskar et al., 2016; Newman et al., 2018) and cave waters (Gaálová et al., 2014).

5.4. Relationship between the bacteria present in the biofilms and air of La Garma Cave

Understanding the relationship between sediment-dwelling and airborne bacteria can provide valuable insights into the cave's microbial dynamics. The colonization of cave sediments by bacteria is often influenced by factors such as texture, chemical and mineral composition, availability of organic matter, and humidity levels. The sediments act as reservoirs, providing habitats for diverse microbial communities (Pfendler et al., 2018).

Airborne bacteria in caves can originate from various sources. Some bacteria may be directly introduced from external environments (atmosphere, soils, plants, etc.), carried by air currents, or via the activities of organisms that inhabit or are transported by visitors to the cave. Others may originate from the cave itself, released into the air from disturbed sediments and other substrata (Saiz-Jimenez, 2015). In fact, bacteria present in the biofilms colonizing the sediments may become aerosolised and suspended in the air, allowing them to disperse and potentially colonise new cave areas and substrata. Conversely, airborne bacteria can settle on the cave sediments, leading to their colonization.

The pattern of bacterial dispersion in La Garma Cave is clearly influenced by the air circulation in the subterranean ecosystem. Considering the three seasonal samplings as a whole, the highest concentrations of airborne bacteria were found at the end of summer, coinciding with the stage of intense ventilation pulses in the cave that also provoke the sudden and intense increments of suspended particles. Individualised by seasons, a defined pattern in the behavior of the bacteria was not observed other than the abundant occurrence of *Streptomyces* species in all seasons, accompanied by *Pseudomonas* in the ventilation stage, *Arthrobacter*-related genera in ventilation pulses and *Bacillus*-related genera in the stagnation stage. The presence of all these genera of bacteria is common in caves and points out that their abundance is mainly related to the capacity to produce spores that are easily transported by air.

Unlike airborne fungi, with notable differences between ventilation and stagnation periods (Sanchez-Moral et al., 2021), the dynamics of airborne bacteria do not have a seasonal basis, although the maximum bacterial concentration outside did coincide with the maximum mean value inside the cave. Both maxima were registered during sudden and intense pulses of ventilation triggered by southerly winds outside. This could be because all airborne fungi occurred as spores, easily transported by air flows and cultivable, while airborne bacteria were composed of spore-forming taxa (most likely originated from cave dwellers and biofilms), besides the bacteria attached to dust particles randomly removed by visitors and/or sporadic archaeological activities. In addition, a large number of cave bacteria are not cultivable in the laboratory (Cuezva et al., 2012; Porca et al., 2012; Jurado et al., 2020; Martin-Pozas et al., 2020).

An important feature of La Garma Cave is the progressive bacterial colonization on walls, and sediments across the cave with the formation of extensive biofilms areas (Figures 1 and 3), a process that has been observed over the last two decades. Natural cave aerodynamics plays a key role in bacterial dispersion, both because of the seasonal cave air renewal or through the sudden ventilation pulses linked to the peak meteorological phenomena such as warm southerly winds in the exterior. During early and mid-summer, the cave air renewal affects the upper galleries through the inlet of warmer outside air into the cooler cave environment, triggering an outstanding condensation phenomenon at the beginning of the Middle Gallery and its connection with the Upper Gallery. In those areas with high rates of condensation, an intense development of microbial colonization is observed that progressively advances towards the internal zone of the Middle Gallery (Sector IV and beyond; Figure 1A).

Human-induced disruption of the cave environment was also noticed from the results obtained by our microbiological monitoring. Thus, sampling on the biofilms of sediments and cultivation resulted in the isolation of a few of the most abundant airborne taxa, including; *Streptomyces cyaneofuscatus*, *Streptomyces pratensis*, *Streptomyces avidinii*, *Peribacillus simplex*, *Bacillus licheniformis*, *Rhodococcus erythropolis* and

Stenotrophomonas maltophilia. This close relationship between biofilms microbiome and airborne bacteria points that likely the sediment profiles excavated in the archaeological investigations were readily colonised by airborne bacteria. Conversely, some of the most abundant bacteria reported in Figure 4 are potentially released from sediments surfaces into the air by human activities (e.g. archaeological excavations, footsteps of visitors who removed the biofilms covering the ground sediments, etc.).

6. Concluding remarks: Implications for decision-making and sustainable cave management

The presented microclimate and microbiological monitoring protocol offers a comprehensive approach to the preventive conservation of rock-art caves. Its application to the World Heritage Site La Garma Cave has shed light on the cave's specific vulnerabilities and preservation needs, providing evidence-based strategies for sustainable management. In the case of La Garma Cave, a spatial basis making-decision on the cave management strategies was based on the microclimatic disturbance analysis of key parameters (temperature, relative humidity, CO_2, wind speed), airborne particles and composition and bacteria present in the biofilms and cave air.

The data set obtained allowed us to understand that this complex karst system, with galleries at various levels interconnected by vertical shafts, exhibits areas with significantly distinct environmental characteristics and therefore varying degrees of vulnerability. While the entire system would be affected by visitor entry, areas with a more active aerodynamic regime, such as the Upper Gallery and the Middle Gallery, would experience a significantly lower impact compared to areas with greater environmental stability, such as the Lower Gallery, where irreversible deterioration could occur in a short period of time. Through a detailed analysis of the data gathered from this most stable Lower Gallery, particularly in Sectors I and IV, which house the most representative areas with exceptional Palaeolithic settlements, conclusions can be drawn that are applicable to other cave sectors with similar aerodynamic and morphological conditions:

- Sector I is a low-energy area with very low thermal oscillation ranges and a high degree of isolation from the air circulation regime that connects the upper levels with the Lower Gallery. Analysis of microclimatic disruptions resulting from human presence indicates that despite the small group sizes (3-4 individuals), this area remains fragile and prone to cumulative effects. Moreover, the morphology of Sector I, characterized by lower ceilings, renders it more susceptible to impacts and the persistence of cumulative effects, which could potentially alter its current environmental stability. The fragility and vulnerability of Sector I make it crucial to consider conservation strategies and prevent the modification of its current environmental stability.

- The results of the microclimatic disturbance analysis in Sector IV indicate that the presence of visitors had slight or undetectable effects on microclimatic parameters, such as temperature, CO_2 concentration, relative humidity, and wind speed. The lower gallery morphology in Sector IV, with a narrow section and high ceilings, directed the impacts away from the floor and painting panels, where monitoring equipment is located, resulting in moderate and lower-range impacts compared to natural dynamics and did not generate cumulative effects. However, human transit in Sector IV caused intense resuspension of coarse particles, unlike the natural dynamics. Active remobilization of soil particles caused by the passage of visitors and their subsequent redepositing can result in blackening of walls and floors, a redistribution of cave-dwelling microorganisms, and in this area, have a direct impact on the main panel of the Palaeolithic paintings, making it one of the primary factors contributing to deterioration.

- In the Upper and Middle Gallery, where there is a much more active aerodynamic regime, the impact of visitors on temperature, humidity, and CO_2 concentration remains within the levels of natural variability. However, these areas, characterised by greater exchange of matter and energy with the external environment, exhibit a higher susceptibility to microbial colonisation. This makes them potential sources of microorganism dissemination into the more stable interior areas. In this context, the ease with which bacteria colonise the surface of fresh sediments, visible to the naked eye during archaeological excavations (as shown in Figure 2E), underscores the close connection and composition similarities between the microbiome of biofilms and airborne bacteria. In particular, the most abundant airborne bacteria are those capable of spreading through spores.

This illustrates the importance of assessing the extent of microbial colonisation in non-tourist caves and understanding the bioclimatic variables that determine the colonisation and dissemination of microorganisms in the subterranean environment. Such evaluations are essential to quantify their vulnerability to both the presence of humans and natural air renewal processes. They should be taken into account when establishing conservation strategies in anticipation of increased visitor activity or tourist adaptations.

The common strategy for cave conservation should focus on minimising the influx of nutrients into the cave, maintaining a stable internal environment, and minimising the exchange of matter and energy with the outside. This can be summarised as the prevention of actions that may lead to the release of suspended particles or bacterial cells into the air. However, the results suggest that a single conservation strategy may not be suitable for all cave sectors. Instead, preventive or corrective measures should be adapted to each sector based on its particular bioclimatic characteristics.

Therefore, following the study, the proposed conservation strategy for cave managers includes the following specific measures:

- Managing the external environment of the cave is crucial for its preservation. When defining protection strategies, it is essential to take into account the geological features that govern the flow of matter and energy in the karst system, as these features can potentially impact the hypogeal ecosystem (Elez et al., 2013). It is imperative to avoid any alterations to the natural entrances of the cave or modifications to the surrounding vegetation cover, as these actions are likely to disrupt or intensify the natural ventilation processes within the cave, as described above.

- Adapting this cave for tourist visits is not advisable, as visitor entry into the cavity would entail a dual risk: mechanical (breakage and displacement of archaeological remains and speleothem formations), and bioclimatic (disturbance of thermo-hygrometric stability in certain areas of the cavity and potential dispersal of airborne microorganisms). In the case of access to the Lower Gallery, it should be limited to individuals essential for research and maintenance purposes.

- Continue monitoring of the microclimatic and airborne particles in the cave, paying particular attention to the most stable areas, such as Sector I of the Lower Level, and the more vulnerable areas, such as Sector IV with the painting panel. Additionally, monitor locations with different ventilation patterns, even those with opposing patterns, to better understand the cave's response to external meteorological changes.

- Microorganisms are not only present and active in areas where they form visible colonies, but can also thrive in high quantities with significant rates of metabolic activity in areas where they do not form visible colonies. It is highly recommended to conduct a study to measure biomarkers such as ATP in sediments throughout the entire cave to assess the extent of the presence of microbes in areas where colonies are not yet visible. Recent studies have demonstrated that the integration of biomarkers such as ATP into environmental monitoring can substantially improve the methods used to investigate the detrimental impacts of tourism on cave ecosystems (Mulec et al., 2016; Martin-Pozas et al., 2023).

- One of the essential steps for preventive conservation is the strategic installation of steel walkways with anticorrosion treatment and non-slip design throughout the transit areas inside the cave, especially before beginning any archaeological research. This is an effective measure to prevent the resuspension of particles and, as a result, reduce the risk of bacterial dispersion.

- In order to minimise the impacts of archaeological research, in addition to installing metal walkways, the following measures are recommended:

- Minimise the removal of materials and sediments from the Middle and Lower Gallery, especially in Sector I, where residence times should be strictly controlled.
- Stablish a strict control over clothing and footwear changes for all individuals accessing the cavity, even for short visits.
- Temporarily covering surfaces near the work area with a geotextile material that acts as a protective membrane. The aim is for this protective membrane to capture most of the suspended particulate matter during the research period and then remove it.
- If research coincides with the predominant ventilation season, particularly during periods of sudden ventilation pulses, consider installing a temporary geotextile-type protective barrier at the cave entrance (La Garma A) to limit the entry of external materials. This requires a mandatory assessment of its effectiveness through detailed monitoring of suspended particles, airborne microorganisms, and other microclimatic parameters within the vicinity of this barrier.

The microclimate, airborne particles and microbiological monitoring approach addressed in this study serves as a valuable model for the conservation of similar World Heritage subterranean sites and, hence, its application would contribute to the long-term safeguarding of our global cultural heritage.

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References

AEMET-IM, 2011. Iberian Climate Atlas. Air temperature and precipitation (1971-2000) in Agencia Estatal de Metereologia (España), Instituto de Metereologia (Portugal) (Eds.). https://doi.org/10.31978/784-11-002-5

Arias, P., Ontañón, R., 2012. La Garma (Spain): Long-term human activity in a karstic system, in: Bergsvik, K.A., Skeates, R. (Eds.), Caves in context. Oxbow Books, Oxford. pp. 101-117. https://doi.org/10.13140/2.1.2849.0248

Azua-Bustos, A., Gonzalez-Silva, C., Fernandez-Martínez, M.A., Arenas-Fajardo, C., Fonseca, R., Martin-Torres, F.J., Fernandez-Sampedro, M., Fairen, A.G., Zorzano, M-P., 2019. Aeolian transport of viable microbial life across the Atacama Desert, Chile: Implications for Mars. Sci. Rep. 9, 11024-11024. https://doi.org/10.1038/s41598-019-47394-z

Baker, A., Genty, A., 1998. Environmental pressures on conserving cave speleothems: effects of changing surface land use and increased cave tourism. J. Environ. Manage. 53(2), 165-175. https://doi.org/10.1006/jema.1998.0208

Baldini, L.M., McDermott, F., Baldini, J.U.L., Arias, P., Cueto, M., Fairchild, I.J., Hoffmann, D.L., Mattey, D.P., Mueller, W., Nita, D.C., Ontanon, R., Garcia-Monco, C., Richards, D.A., 2015. Regional temperature, atmospheric circulation, and sea-ice variability within the younger dryas event constrained using a speleothem from northern iberia. Earth Planet. Sci. Lett. 419, 101-110. https://doi.org/10.1016/j.epsl.2015.03.015

Banskar, S., Bhute, S.S., Suryavanshi, M.V., Punekar, S., Shouche, Y.S., 2016. Microbiome analysis reveals the abundance of bacterial pathogens in Rousettus leschenaultii guano. Sci. Rep. 6, 36948-36948. https://doi.org/10.1038/srep36948

Belyagoubi L., Belyagoubi-Benhammou N., Jurado V., Dupont J., Lacoste S., Djebbah F., Ounadjela F.Z., Benaissa S., Habi S., Abdelouahi D.E., Saiz-Jimenez C., 2018. Antimicrobial activities of culturable microorganisms (actinomycetes and fungi) isolated from Chaabe Cave, Algeria. Int. J. Speleol. 47, 189-199. https://doi.org/10.5038/1827-806X.47.2.2148

Brenner, D.J.,Farmer III, J.J., 2005. Family I. Enterobacteriaceae, in: Brenner, D.J., Krieg, N.R., Staley, J.T., Garrity, G.M., Boone, D.R., Vos, P., Goodfellow, M., Rainey, F.A., Schleifer, K.H. (Eds.), Bergey's Manual of Systematic Bacteriology. Springer, New York, pp. 587–607.

Bontemps, Z., Alonso, L., Pommier, T., Hugoni, M., Moënne-Loccoz, Y., 2022. Microbial ecology of tourist Palaeolithic caves. Sci. Total Environ. 816, 151492-151492. https://doi.org/10.1016/j.scitotenv.2021.151492

Busse, H.J., 2016. Review of the taxonomy of the genus Arthrobacter, emendation of the genus Arthrobacter sensu lato, proposal to reclassify selected species of the genus Arthrobacter in the novel genera Glutamicibacter gen. nov., Paeniglutamicibacter gen. nov., Pseudoglutamicibacter gen. nov., Paenarthrobacter gen. nov. and Pseudarthrobacter gen. nov., and emended description of Arthrobacter roseus. Int. J. Syst. Evol. Microbiol. 66, 9-37. https://doi.org/10.1099/ijsem.0.000702

Calaforra, J.M., Fernandez-Cortes, A., 2005. Geotourism in Spain: resources and environmental management, in: R. Dowling, R., Newsome, D. (Eds.), Geotourism. Elservier Butterworth-Heinemann, Oxford (UK), pp.199-220.

Cuezva, S., Fernandez-Cortes, A., Porca, E., Pasic, L., Jurado, V., Hernandez-Marine, M., Serrano-Ortiz, P., Cañaveras, J.C., Sanchez-Moral, S., Saiz-Jimenez, C., 2012. The biogeochemical role of Actinobacteria in Altamira Cave, Spain. FEMS Microbiol. Ecol. 81, 281-290. https://doi.org/10.1111/j.1574-6941.2012.01391.x

Comas-Bru, L., McDermott, F., 2015. Data-model comparison of soilwater δ18O at a temperate site in N. Spain with implications for interpreting speleothem δ18O. J. Hydrol. 530, 216-224. https://doi.org/10.1016/j.jhydrol.2015.09.053

Constantin, S., Mirea, I.C., Petculescu, A., Arghir, R.A., Măntoiu, D.Ș., Kenesz, M., Robu, M., Moldovan, O.T., 2021. Monitoring Human Impact in Show Caves. A Study of Four Romanian Caves. Sustainability 13, 1619. https://doi.org/10.3390/su13041619

Cuezva, S., Fernandez-Cortes, A., Jurado, V., Saiz-Jimenez, C., Ontañon, R., Arias, P., Hernandez-Vicente, I., Sanchez-Moral, S., 2016. Investigación aplicada a la conservación preventiva del sistema kárstico de La Garma (Omoño, Ribamontán al Monte, Cantabria), in: Andreo, B., Durán, J.J. (Eds.), El Karst y el Hombre: las Cuevas como Patrimonio Mundial Asociación de Cuevas Turísticas Españolas, Madrid, pp. 307-318

De Mandal, S., Sanga, Z., Kumar N.S., 2015. Metagenome sequencing revealed Rhodococcus dominance in Farpuk Cave, Mizoram, India, an eastern Himalayan biodiversity hot spot region. Genome Announc. 3(3), e00610-15. https://doi.org/10.1128/genomeA.00610-15

Dominguez-Moñino, I., Jurado, V., Gonzalez-Pimentel, J.L., Miller, A.Z., Hermosin, B., Saiz-Jimenez, C., 2018. Bacillus onubensis sp. nov., isolated from the air of two Andalusian caves. Syst. Appl. Microbiol., 41, 167-172. https://doi.org/10.1016/j.syapm.2018.01.001

Dominguez-Moñino, I., Jurado, V., Rogerio-Candelera, M.A., Hermosin, B., Saiz-Jimenez, C., 2021. Airborne bacteria in show caves from Southern Spain. Microb. Cell, 8, 247-255. https://doi.org/10.15698/mic2021.10.762

Echigo, A., Hino, M., Fukushima, T., Mizuki, T., Kamekura, M., Usami, R., 2005. Endospores of halophilic bacteria of the family Bacillaceae isolated from non-saline Japanese soil may be transported by Kosa event (Asian dust storm). Saline syst. 1, 8-8. https://doi.org/10.1186/1746-1448-1-8

Elez, J., Cuezva, S., Fernandez-Cortes, A., Garcia-Anton, E., Benavente, D., Canaveras, J.C., Sanchez-Moral, S., 2013. A GIS-based methodology to quantitatively define an Adjacent Protected Area in a shallow karst cavity: The case of Altamira cave. J. Environ. Manage. 118, 122-134. https://doi.org/10.1016/j.jenvman.2013.01.020

Enyedi, N.T., Makk, J., Kótai, L., Berényi, B., Klébert, S., Sebastyén, Z., Molnár, Z., Borsodi, A.K., Leél-Össy, S., Demény, A., Németh, P., 2020. Cave bacteria-induced amorphous calcium carbonate formation. Sci. Rep. 10, 8696-8696. https://doi.org/10.1038/s41598-020-65667-w

Fernandez-Cortes, A., Calaforra, JM., Jimenez-Espinosa, R., Sanchez-Martos, F., 2006. Geostatistical spatiotemporal analysis of air temperature as an aid to delineating thermal stability zones in a potential show cave: Implications for environmental management. J. Environ. Manage. 81(4), 371-383. https://doi.org/10.1016/j.jenvman.2005.11.011

Fernandez-Cortes, A., Cuezva, S., Sanchez-Moral, S., Porca, E., Jurado, V., Saiz-Jimenez, C., 2011. Detection of human-induced environmental disturbances in a show cave. Environ. Sci. Pollut. Res. 18, 1037-1045. https://doi.org/10.1007/s11356-011-0513-5

Fernandez-Cortes, A., Benavente, D., Cuezva, S., Cañaveras, J.C., Alvarez-Gallego, M., Garcia-Anton, E., Soler, V., Sanchez-Moral, S., 2013. Effect of water vapour condensation on the radon content in subsurface air in a hypogeal inactive volcanic environment in Galdar cave, Spain, Atmos. Environ. 75, 15–23. https://doi.org/10.1016/j.atmosenv.2013.04.030.

Gaálová, B., Donauerová, A., Seman, M., Bujdáková, H., 2014. Identification and ß-lactam resistance in aquatic isolates of Enterobacter cloacae and their status in microbiota of Domica Cave in Slovak Karst (Slovakia). Int. J. Speleol. 43, 69-77. https://doi.org/10.5038/1827-806X.43.1.7

Garcia-Anton, E., Cuezva, S., Jurado, V., Porca, E., Miller, A.Z., Fernandez-Cortes, A., Saiz-Jimenez, C., Sanchez-Moral, S., 2014. Combining stable isotope ($\delta^{13}C$) of trace gases and aerobiological data to monitor the entry and dispersion of microorganisms in caves. Environ. Sci. Pollut. Res. 21, 473-484. https://doi.org/10.1007/s11356-013-1915-3

Gonzalez-Pimentel, J.L., Jurado, V., Hermosin, B., Saiz-Jimenez, C., 2020. Draft genome sequences of two Streptomyces strains, MZ03-37T and MZ03-48, isolated from lava tube speleothems. Microbiol. Resour. Announc. 9 (25), e00576-20. https://doi.org/10.1128/MRA.00576-20

Harley, GL., Polk, JS., North, LA., Reeder, PP., 2011. Application of a cave inventory system to stimulate development of management strategies: The case of west-central Florida, USA. J. Environ. Manage. 92(10), 2547-2557. https://doi.org/ 10.1016/j.jenvman.2011.05.020

Heaton, T., 1986. Caves: a tremendous range in energy environments in earth. National Speleological Society News, 44(8), 301–304.

Hoyos, M., Soler, V., Cañaveras, J.C., Sanchez-Moral, S., Sanz-Rubio E., 1998. Microclimatic characterization of a karstic cave: human impact on microenvironmental parameters of a prehistoric rock art cave (Candamo Cave, northern Spain). Environ. Geol., 33, 321–242. https://doi.org/10.1007/s002540050242

Jones, A.L., Goodfellow, M., 2012. Genus IV. Rhodococcus (Zopf 1891) emend. Goodfellow, Alderson and Chun 1998a, in: Goodfellow, M., Kämpfer, P., Busse, H.J., Trujillo, M.E., Suzuki, K., Ludwig, W., Whitman, W.B. (Eds.), Bergey's Manual of Systematic Bacteriology, The Actinobacteria. Springer, New York, pp. 437-464.

Jurado, V., Laiz, L., Rodriguez-Nava, V., Boiron, P., Hermosin, B., Sanchez-Moral, S. & Saiz-Jimenez, C., 2010. Pathogenic and opportunistic microorganisms in caves Int. J. Speleol. 39, 15-24. https://doi.org/10.5038/1827-806X.39.1.2

Jurado, V., Laiz, L., Sanchez-Moral, S., Saiz-Jimenez, C., 2014. Pathogenic microorganisms related to human visits in Altamira Cave, Spain. in: Saiz-Jimenez, C. (Ed.), The Conservation of Subterranean Cultural Heritage. CRC Press/Balkema, Leiden, pp. 229-238.

Jurado, V., del Rosal, Y., Gonzalez-Pimentel, J.L., Hermosin, B., Saiz-Jimenez, C., 2020. Biological control of phototrophic biofilms in a show cave: The case of Nerja Cave. Appl. Sci., 10, 3448-3448. https://doi.org/10.3390/app10103448

Jurado, V., del Rosal, Y., Jimenez de Cisneros, C., Liñán, C., Martin-Pozas, T., Gonzalez-Pimentel, J.L., Hermosin, B., Saiz-Jimenez, C., 2022. Microbial communities in carbonate precipitates from drip waters in Nerja Cave, Spain. PeerJ, 10, 13399-13399. https://doi.org/10.7717/peerj.13399

Juretschko, S., Timmermann, G., Schmid, M., Schleifer, K.H., Pommering-Röser, A., Koops, H.P., Wagner, M., 1998. Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: Nitrosococcus mobilis and Nitrospira-like bacteria as dominant populations. Appl. Environ. Microbiol. 64, 3042-3051. https://doi.org/10.1128/AEM.64.8.3042-3051.1998

Köck, R., Daniels-Haardt, I., Becker, K., Mellmann, A., Friedrich, A.W., Mevius, D., Schwarz, S., Jurke, A., 2018. Carbapenem-resistant Enterobacteriaceae in wildlife, food-producing, and companion animals: a systematic review. Clin. Microbiol. Infect. 24, 1241-1250. https://doi.org/10.1016/j.cmi.2018.04.004

Kottek, M., Grieser, J., Beck, C., Rudolf, B., Rubel, F., 2006. World Map of the Köppen-Geiger climate classification updated. Meteorologische Zeitschrift 15(3), 259-263. https://doi.org/10.1127/0941-2948/2006/0130

Laiz, L., Groth, I., Gonzalez, I., Saiz-Jimenez, C., 1999. Microbiological study of the dripping waters in Altamira cave (Santillana del Mar, Spain). J. Microbiol. Methods. 36, 129-138. https://doi.org/10.1016/s0167-7012(99)00018-4

Lan, X., Tans, P., Thoning, K.W., 2023. Trends in globally-averaged CO2 determined from NOAA Global Monitoring Laboratory measurements. Version 2023-10. https://doi.org/10.15138/9N0H-ZH07

Lobo, H. A. S., Trajano, E., Marinho, M. d. A., Bichuette, M. E., Scaleante, J. A. B., Scaleante, O. A. F., et al., 2013. Projection of tourist scenarios onto fragility maps: Framework for determination of provisional tourist carrying capacity in a Brazilian show cave. Tour. Manag. 35, 234-243. https://doi.org/10.1016/j.tourman.2012.07.008

Martin-Pozas, T., Sanchez-Moral, S., Cuezva, S., Jurado, J., Saiz-Jimenez, C., Perez-Lopez, R., Carrey, R., Otero, N., Giesemann, A., Well, R., Calaforra, J.M., Fernandez-Cortes, A., 2020. Biologically mediated release of endogenous N_2O and NO_2 gases in a hydrothermal, hypoxic subterranean environment. Sci. Total Environ. 747, 141218-141218. https://doi.org/10.1016/j.scitotenv.2020.141218

Martin-Pozas, T., Fernandez-Cortes, A., Cuezva, S., Cañaveras, J.C., Benavente, D., Duarte, E., Saiz-Jimenez, C., Sanchez-Moral, S., 2023. New insights into the structure, microbial diversity and ecology of yellow biofilms in a Palaeolithic rock art cave (Pindal Cave, Asturias, Spain). Science of the Total Environment 897, 165218. http://dx.doi.org/10.1016/j.scitotenv.2023.165218

Martin-Pozas, T., Cuezva, S., Fernandez-Cortes, A., Benavente, D., Saiz-Jimenez, C., Sanchez-Moral, S. 2023. Prokaryotic communities inhabiting a high-radon subterranean ecosystem (Castañar Cave, Spain): Environmental and substrate-driven controls. Microbiological Research, 277, 12751. https://doi.org/10.1016/j.micres.2023.127511.806X.45.3.1987.

Martin-Sanchez P.M., Jurado V., Porca E., Bastian F., Lacanette D., Alabouvette C., Saiz-Jimenez C., 2014. Airborne microorganisms in Lascaux Cave (France). Int. J. Speleol. 43, 295-303. https://doi.org/10.5038/1827-806X.43.3.6

Miller, A.Z., García-Sánchez, A.M., Coutinho, M.L., Pereira, M.F.C., Gázquez, F., Calaforra, J.M., Forti, P., Martínez-Frías, J., Toulkeridis, T., Caldeira, A.T., Saiz-Jimenez, C., 2020. Colored microbial coatings in show caves from the Galapagos Islands (Ecuador): First microbiological approach. Coatings ,10, 1134-1134. https://doi.org/10.3390/coatings10111134.

Moldovan, O.T., Bercea, S., Nastase-Bucur, R., Constantin, S., Kenesz, M., Mirea, I.C., Petculescu, A., Robu, M., Arghir, R.A., 2020. Management of water bodies in show caves – A microbial approach. Tour. Manag. 78, 104037-104037. https://doi.org/10.1016/j.tourman.2019.104037

Mulec, J., Oarga-Mulec, A., 2016. ATP luminescence assay as a bioburden estimator of biomass accumulation in caves. Int. J. Speleol. 45, 207–218. https://doi.org/ 10.5038/1827.

Mulec, J., Oarga-Mulec, A., Sturm, S., Tomazin, R., Matos, T., 2017. Spacio-temporal distribution and tourist impact on airborne bacteria in a cave (Škocjan Caves, Slovenia). Diversity, 9, 28-28. https://doi.org/10.3390/d9030028

Newman, M.M., Kloepper, L.N., Duncan, M., McInroy, J.A., Kloepper, J.W., 2018. Variation in bat guano bacterial community composition with depth. Front. Microbiol. 9, 914-914. https://doi.org/10.3389/fmicb.2018.00914

Ontanon, R., 2003. Sols et structures d'habitat du Paleolithique superieur, nouvelles donnees depuis les Cantabres: La Galerie Inferieure de La Garma (Cantabrie, Espagne). Anthropologie, 107, 333–363. https://doi.org/10.1016/S0003-5521(03)00037-2

Otlewska, A., Gutarowska, B., 2016. Environmental parameters conditioning microbially induced mineralization under the experimental model conditions. The journal of the Polish Academy of Sciences, 63, 343-351. https://doi.org/10.18388/abp.2015_1172

Patel, S., Gupta, R.S., 2020. A phylogenomic and comparative genomic framework for resolving the polyphyly of the genus Bacillus: Proposal for six new genera of Bacillus species, Peribacillus gen. nov., Cytobacillus gen. nov., Mesobacillus gen. nov., Neobacillus gen. nov., Metabacillus gen. nov. and Alkalihalobacillus gen. nov. Int. J. Syst. Evol. Microbiol. 70, 406-438. https://doi.org/10.1099/ijsem.0.003775

Peñalver, E., Alvarez-Fernandez, E., Arias, P., Delclos, X, Ontañon, R., 2007. Local amber in a Palaeolithic context in Cantabrian Spain: the case of La Garma A. J. Archaeol. Sci. 34, 843-849. https://doi.org/10.1016/j.jas.2006.03.006

Pfendler, S., Karimi, B., Maron, P.-A., Ciadamidaro, L., Valot, B., Bousta, F., Alaoui-Sosse, B., Alaoui-Sosse, B., Aleya, L., 2018. Biofilm biodiversity in French and Swiss show caves using the metabarcoding approach: First data. Sci. Total Environ. 615, 1207-1217. https://doi.org/10.1016/j.scitotenv.2017.10.054

Piano, E., Biagioli, F., Nicolosi, G., Coleine, C., Poli, A., Prigione, V., Zanellati, A., Addesso, R., Varese, G.C., Selbmann, L., Isaia, M., 2023. Tourism affects microbial assemblages in show caves. Sci. Total Environ. 871, 162106-162106. https://doi.org/10.1016/j.scitotenv.2023.162106

Porca-Belio, E., 2011. Aerobiología: mecanismos de dispersión de los microorganismos en cuevas turísticas. Ph. D. Thesis, University of Seville.

Porca, E., Jurado, V., Martin-Sanchez, P.M., Hermosin, B., Bastian, F., Alabouvette, C., Saiz-Jimenez, C., 2011. Aerobiology: An ecological indicator for early detection and control of fungal outbreaks in caves. Ecol. Indic. 11, 1594-1598. https://doi.org/10.1016/j.ecolind.2011.04.003

Porca, E., Jurado, V., Zgur-Bertok, D., Saiz-Jimenez, C., Pasic, L., 2012. Comparative analysis of yellow microbial communities growing on the walls of geographically distinct caves indicates a common core of microorganisms involved in their formation. FEMS Microbiol. Ecol. 81, 255-266. https://doi.org/10.1111/j.1574-6941.2012.01383.x

Rao, Y., Li, H., Chen, M., Fu, Q., Zhuang, G., Huang, K., 2020. Characterization of Airborne Microbial Aerosols during a Long-range Transported Dust Event in Eastern China: Bacterial community, Influencing Factors, and Potential Health Effects. Aerosol. Air. Qual. Res. 20, 2834-2845. https://doi.org/10.4209/aaqr.2020.01.0030

Riquelme, C., Dapkevicius, M.D.L.E., Miller, A.Z., Charlop-Powers, Z., Brady, S., Mason, C., Cheeptham, N., 2017. Biotechnological potential of Actinobacteria from Canadian and Azorean volcanic caves. Appl. Microbiol. Biotechnol. 101, 843-857. https://doi.org/10.1007/s00253-016-7932-7

Rudzka-Phillips, D., McDermott, F., Jackson, A., Fleitmann, D., 2013. Inversemod-elling of the C-14 bomb pulse in stalagmites to constrain the dynamics of soil carbon cycling at selected European cave sites. Geochim. Cosmochim. Acta 112, 32–51. https://doi.org/10.1016/j.gca.2011.05.022

Russell, MJ and MacLean, VL., 2008. Management issues in a Tasmanian tourist cave: Potential microclimatic impacts of cave modifications. J. Environ. Manage. 87(3), 474-483. https://doi.org/10.1016/j.jenvman.2007.01.012

Rusznyák, A., Akob, D.M., Nietzsche,S., Eusterhues, K., Totsche, K.U., Neu, T.R., Frosch, T., Popp, J., Keiner, R., Geletneky, J., Katzschmann, L., Schulze, E-D., Küsel, K., 2012. Calcite Biomineralization by Bacterial Isolates from the Recently Discovered Pristine Karstic Herrenberg Cave. Appl. Environ. Microbiol. 78, 1157-1167. https://doi.org/10.1128/AEM.06568-11

Saiz-Jimenez, C., 2015. The Microbiology of Show Caves, Mines, Tunnels and Tombs: Implications for Management and Conservation, in: Engel, A.S. (Ed.), Microbial Life of Cave Systems. De Gruiter: Berlin, Germany, pp. 231–261.

Saiz-Jimenez, C., Cuezva, S., Jurado, V., Fernandez-Cortes, A., Porca, E., Benavente, D., Cañaveras, J.C., Sanchez-Moral, S., 2011. Palaeolithic Art in Peril: Policy and Science Collide at Altamira Cave. Science, 334, 42-43. https://doi.org/10.1126/science.1206788

Sanchez-Moral, S., Soler, V., Cañaveras, J.C., Sanz-Rubio, E., Van Grieken, R., Gysels, K., 1999. Inorganic deterioration affecting the Altamira Cave, N Spain: quantitative approach to wal lcorrosion (solutional etching) processes induced by visitors. The Science of the Total Environment 243/244, 67–84. https://doi.org/10.1016/S0048-9697(99)00348-4

Sanchez-Moral, S., 2014. Estudio integral del estado de conservación de la cueva de Altamira y su arte paleolítico (2007-2009). Perspectivas futuras de conservación, first edition, Monografías. Museo Nacional y Centro de Investigación de Altamira, Madrid.

Sanchez-Moral, S., Jurado, V., Fernandez-Cortes, A., Cuezva, S., Martin-Pozas, T., Gonzalez-Pimentel, J.L., Ontañon, R., Saiz-Jimenez, C., 2021. Environment‐driven control of fungi in subterranean ecosystems: the case of La Garma Cave (northern Spain). Int. Microbiol. 24, 573-591. https://doi.org/10.1007/s10123-021-00193-x

Saracho, A.C., Haigh, S.K., Hata, T., Soga, K., Farsang, S., Redfern, S.A.T., Marek, E., 2020. Characterisation of $CaCO_3$ phases during strain-specific ureolytic precipitation. Sci. Rep. 10, 10168-10168. https://doi.org/10.1038/s41598-020-66831-y

Schabereiter-Gurtner, C., Saiz-Jimenez, C., Piñar, G., Lubitz, W., Rolleke, S., 2004. Phylogenetic diversity of bacteria associated with Palaeolithic paintings and surrounding rock walls in two Spanish caves (Llonin and La Garma). FEMS Microbiol. Ecol. 47, 235-247. https://doi.org/10.1016/S0168-6496(03)00280-0

Sebela, S., Turk, J., 2014. Natural and anthropogenic influences on the year-round temperatura dynamics of air and water in Postojna show cave, Slovenia. Tour. Manag. 40, 233-243. https://doi.org/10.1016/j.tourman.2013.06.011

Stone, W., Kroukamp, O., Korber, D.R., McKelvie, J., Wolfaardt, G.M., 2016. Microbes at surface-air interfaces: The metabolic harnessing of relative humidity, surface hygroscopicity, and oligotrophy for resilience. Front. Microbiol. 7, 1563-1563. https://doi.org/10.3389/fmicb.2016.01563

Tomova, I., Lazarkevich, I., Tomova, A., Kambourova, M., Vasileva-Tonkova E., 2013. Diversity and biosynthetic potential of culturable aerobic heterotrophic bacteria isolated from Magura Cave, Bulgaria. Int. J. Speleol. 42, 65-76. https://doi.org/10.5038/1827-806X.42.1.8

Wang, W., Ma, Y., Ma; X., Wu, F., Ma, X., An, L., Feng, H., 2012. Diversity and seasonal dynamics of airborne bacteria in the Mogao Grottoes, Dunhuang, China. Aerobiologia, 28, 27-38. https://doi.org/10.1007/s10453-011-9208-0