



Supporting Online Material for

Paleolithic Art in Peril: Policy and Science Collide at Altamira Cave

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Phototrophic microorganisms in Altamira Cave

Increasing phototrophic biomass production in caves is triggered by light and water, as well as by the particular petrophysical and geochemical characteristics of the rock substrata (*S1-S3*). In April 2002, several green spots were detected on the Polychrome Hall ceiling, among the bison paintings (Fig. S1A). The position of these spots on the ceiling corresponded with the placement of artificial lighting lamps installed on the ground. The use of lighting to facilitate painting observation for visitors is a well-known and common problem in caves (*S1, S2*). In addition to the ceiling, the sediments adjacent to the ground's lighting lamps were heavily colonized by phototrophic microorganisms. This led to closure of the cave and a change in the lighting protocol: replacing fixed lamps with flashlights.

Among the cyanobacteria, we identified species of the genera *Cyanothece* and *Cyanobacterium* (Chroococcales), *Oscillatoria*, and *Phormidium* (Oscillatoriales). Species of the genus *Cyanidium* (Rhodophyta) and *Chlamydomonas*, and less frequently *Chlorococcum* (Chlorophyta), were found near the ground lamps, in the sediments. Cyanobacteria and Chlorophyta have been reported to develop in dark heterotrophic conditions (*S4, S5*), and even in the cases with extremely low light, numerous troglobitic cyanobacteria have adapted to the stressful lighting conditions and contributed to most of the phototrophic biomass in caves (*S6*). It was clear that any change in the lighting conditions, as occurred in 2002 in Altamira Cave, would lead to another “maladie verte,” as exemplified in Lascaux Cave in the 1960s (*S1*). The influx of visitors and the increasing use of flashlights must be avoided, as phototrophic microorganisms are present and can still be observed on the Polychrome Hall ceiling (Fig. S1B).

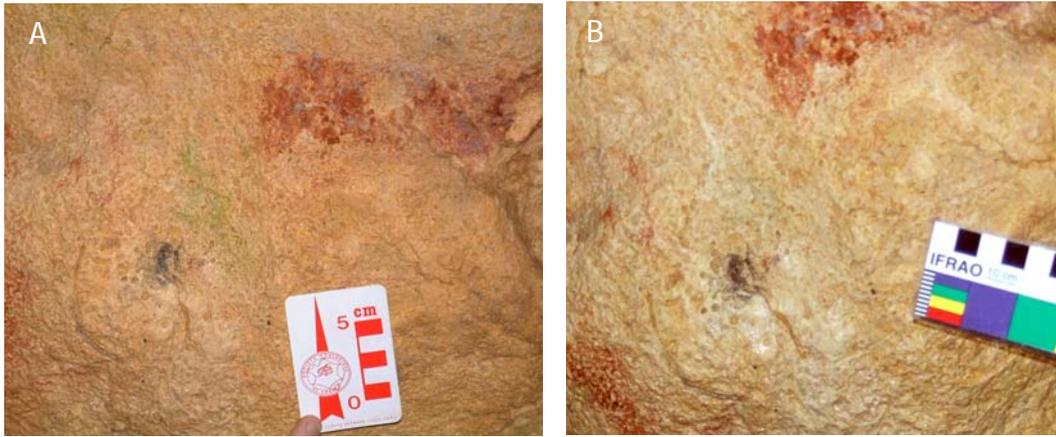


Fig. S1.

Phototrophic microorganisms detected on the Polychrome ceiling. (A) April 2002. (B) March 2011.

Bacteria in Altamira Cave

In Altamira Cave, the areas characterized by higher microenvironmental parameter fluctuations (temperature, relative humidity, carbon dioxide) show a higher variability of microbial communities and lower biomineralization capability. The microbial colonization density decreased progressively toward the interior of the cave and was particularly intense on some areas of the walls and ceiling of the Kitchen Hall. It was characterized by high thermo-hygrometric oscillations in the galleries that access the Polychrome and Wall Halls. In particular, yellow colonies were predominant in the Kitchen Hall (Fig. S2), abundant in the Crossing area, scarce in the Walls Hall and in the gallery that accesses the Polychrome Hall, and nonexistent in the last-named hall (Fig. 1). White colonies were distributed along the cave, from the entrance to the Polychrome Hall, and they are the most abundant in the Polychrome Hall (Fig. S3) and represent the main threat to the paintings because they already colonized the red pigment (hematite). The effect of microbial colonization on hematite was described by Gonzalez et al. (S7). Several studies focused on the characterization of the different bacterial colonies were published elsewhere (S8-S21).

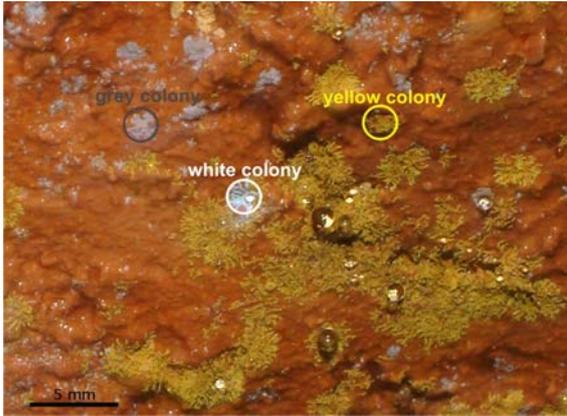


Fig. S2

A wall of the Entrance Hall where the main types of microbial colonies: yellow-, gray- and white-colored can be observed.

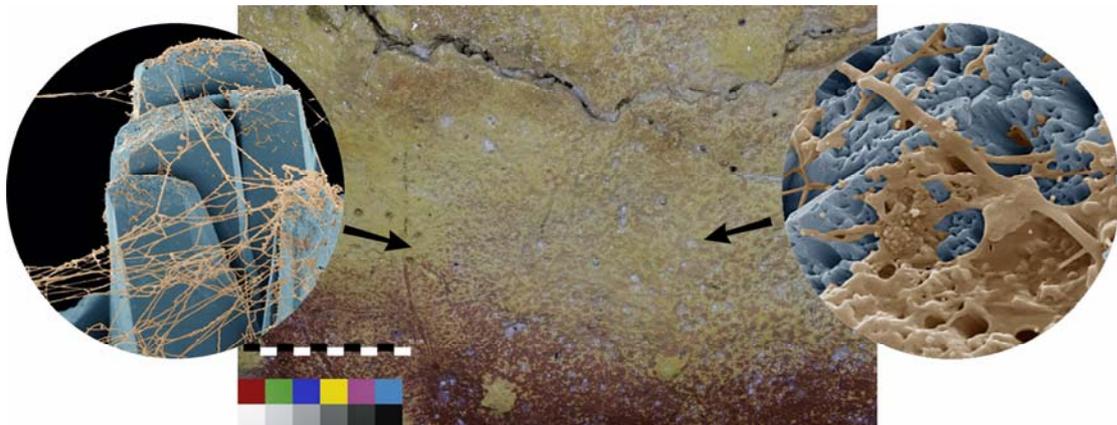


Fig. S3

Microniches generated by corrosion in the Polychrome ceiling. There, the white colonies occupy small voids in the rock, where water is retained more effectively by surface tension forces. **(Right circle)** Artificially colored micrograph of corroded calcite (blue), a preferential habitat for white colonies. Biofilms are represented in brown. **(Left circle)** The presence of microorganisms is also evident in areas where no colonies can be observed by the unaided eye.

Fungi in Altamira Cave

The aerobiology of Altamira Cave was studied on June 3, 2009, and focused on the culturable fraction of fungi present in the air (Table S1). The colony-forming units (CFU)/m³ of all of the fungal spores as a whole was dominated by *Cladosporium* spp. (*Cladosporium* sp., *C. cladosporioides*, *C. cucumerinum*, and *C. macrocarpum*), which reached a total of 80.3% of the spores, followed by *Epicoccum nigrum*, at 14.8%. Other fungal species of *Trichoderma*, *Pochonia*, *Aspergillus*, *Penicillium*, and *Acremonium* accounted for the rest. Outside Altamira Cave, the airborne spores were dominated by *E. nigrum*, *Fusarium* sp., and *Cladosporium* sp. Several studies carried out outdoors showed that the most abundant spore type is *Cladosporium*, representing between 40 and 80% of the total spore numbers in the air (S22). *Aspergillus-Penicillium* type is the most abundant indoors (S23). The abundance of *Cladosporium* in Altamira Cave is not common in other caves previously studied (S24), and these data might be indicative of the strong influence of outdoor fungal spores in the cave air of Altamira and confirm that the main vehicle for transporting fungal spores from the outside to the inside was air. The discovery of *Pinus* and *Zea mays* pollen in the Polychrome Hall also support the effect of transport and dispersal of airborne particles from the exterior air to the interior of the cave. Therefore, the main problem in the conservation of Altamira cave is linked to the entrance door and other connections with the exterior that favor air exchange. In addition, the presence of small animals and insects provides fungus vectors. Fig. S4 shows rodent excrement and a tripod supporting the environmental sensors colonized by fungi. Although rodent excrement is commonly colonized by species of *Phycomyces* and *Mucor* (Fig. S4A), the colonization of metal and plastic instrument bodies is bizarre. The metallic environmental sensors favor vapor condensation, and the tripod feet are conductors of condensed water that ends up in the ground sediments. The condensed and dripped water from the cave contains dissolved organic carbon (S25), which enriched the ground under the tripod leg with nutrients, and these local carbon concentrations enhanced fungal colonization and growth (Fig. S4B). The fungal mycelia were immediately used by collembolans as food, and their excrement contributes to the dispersal of fungi similarly to those reported for Lascaux Cave (S26).



Fig. S4

Fungal growth in Altamira Cave. (A) Rodent excrement. (B) Tripod foot and ground sediment colonized by *Aspergillus* sp.

Table S1.

Aerobiology of Altamira Cave as studied using a SAS sampler. Description of sampling protocol was published elsewhere (S24).

Fungi in the air of Altamira Cave, June 2009		
	ZONE	CFU/m³
Area less influenced by the door	Horse Tail	10
	Well Hall	50
	Great Hall	21
	Hole Hall	10
	Gallery between Hole and Wall Halls	10
Area influenced by the entrance door	Wall Hall	170
	Crossing	330
	End Polychrome Hall	390
	Entrance Polychrome Hall	90
	Entrance gallery	250
	Outdoor	1,750

Environmental study

During the period of microenvironmental monitoring 1997-1999, Altamira Cave was exposed to a restricted visitation schedule, effective from 1982 to September 2002, based on the natural ventilation rates in the Polychrome Hall (S27) and the influence of visitors on temperature, relative humidity, and carbon dioxide (CO₂) concentration (S28-30). One of the main premises was that the entry of visitors did not cause a cumulative effect on the variation of any of these parameters. The regime of visits was established as follows: 11,000 visitors/year, in groups of 5 people plus a guide, a stay of approximately 20 min in the cave and 10 min in the Polychrome Hall, with intervals of about 10 min between consecutive groups. The number of groups per day that entered the cave varied throughout the year; from 2 groups/day (May) to 8 groups/day (June and October). The analysis of the habitual entry and presence of people in the interior of the cave in reference to the microenvironmental balance was done from data corresponding to the annual cycle from February 1997 to January 1998, with 2-min registered intervals. In order to quantify the impacts on the physicochemical conditions caused by the entrance of visitors in the Polychrome Hall, a detailed study was carried out, primarily centered on the increase in air temperature and CO₂ concentration. These essential parameters control the physicochemical processes that occur in a subterranean environment. The anthropogenic impacts on temperature and CO₂ content of air were assessed on 900 visiting groups made up of 6 people each.

Figure S5 shows an example of the impact produced by two consecutive days of visits to the Polychrome Hall in the period of the year (June) in which two unfavorable situations coincided: (i) a greater monthly influx of visitors (8 groups/day, 48 people/day), and (ii) greater thermal similarity between the air mass in the Polychrome Hall and the adjacent areas (crossing and corridor), a fact that favored the exchange of air between both areas through the activation of connective cells (Fig. S6) as a consequence of thermal increases generated by the visits to the Polychrome Hall.

Each group of visitors generated an increase of around +0.14 °C and +110 ppm of CO₂, respectively. In both parameters, the increases per group were cumulative throughout the day and did not recover between consecutive groups, until reaching the daily maximum of 425 ppm CO₂ and 0.28 °C. After a first day of visits in the morning, the atmosphere in the Polychrome Hall was not able to reestablish its initial temperature or CO₂ content conditions, registering an accumulation of 0.01 °C and 75 ppm CO₂ before beginning the following day's series of visits. The air temperature tends to recover the initial conditions more quickly than the CO₂ content. Therefore, in a series of visits from 8 different groups at 10-min intervals, the first 4 groups cause a greater cumulative effect on the temperature (+0.12 °C), whereas the thermal recovery is greater during the last visits of the day.

After a detailed evaluation of the impact that a total of 900 groups had on the microclimate of the Polychrome Hall, increases in average temperature and CO₂ content were quantified per group at 0.07 °C (0.05-0.09 °C) and 51 ppm (23-122 ppm) CO₂, respectively, for a standard 10-min visit.

Each daily series of visiting groups generated an average impact of 0.22°C on the air temperature and 440 ppm on the CO₂ content. The average value of thermal impact caused by each series of daily visits is of the same order of magnitude as the monthly

thermal oscillation range under natural conditions, registered after the closure of the cave to the public in 2002. The greatest CO₂ increases generated by visitor's entry were registered during the periods of greatest thermal stability in the subterranean atmosphere (May-June and November-December).

The average time needed to restore the previous microclimatic conditions from a series of daily visits was quantified at 17 hours for the air temperature. In the case of the CO₂ levels in the air, the recovery time was even greater, although it was difficult to quantify owing to the natural oscillations experienced by concentrations of this gas. The results obtained suggest that once the daily series of visits exceeded a cumulative 70 min, the CO₂ of anthropogenic origin would begin to be assimilated by the interior atmosphere. This phenomenon could indicate the transformation of CO₂ in gas phase into CO₂ dissolved in liquid phase (dripping and condensed waters). The kinetics of this chemical reaction requires about 5 min (S29), and once begun, the water acquires the characteristics of a dissolvent, beginning the corrosion processes on the composition of the carbonated surfaces. The pitches generated by these corrosion processes are niches preferable for the establishment and development of microbial colonies (Fig. S3).

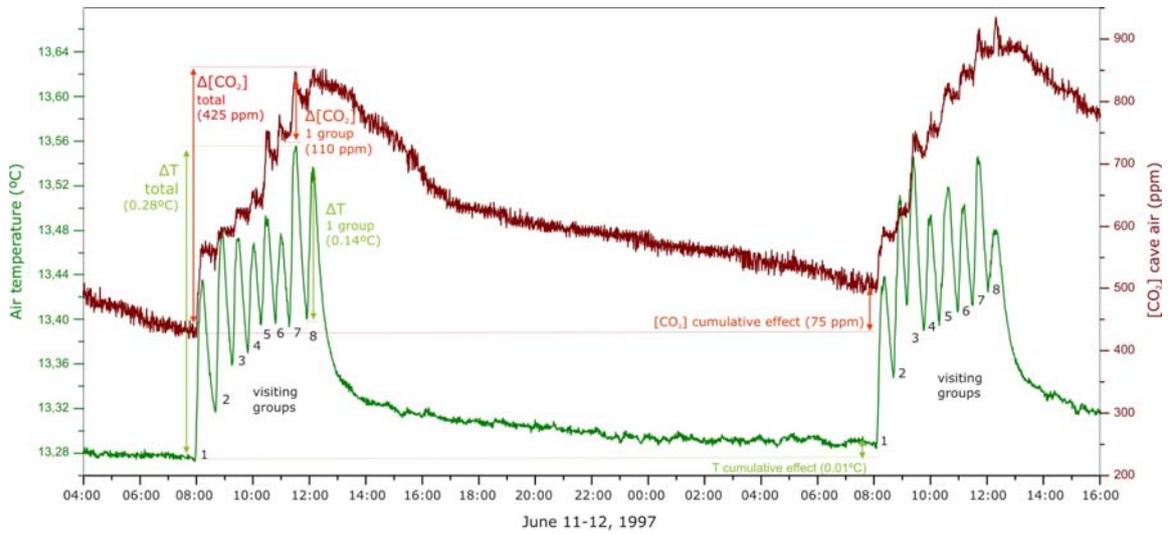


Fig. S5

Microclimatic disturbance in the Polychrome Hall due to several visiting groups during two consecutive days, corresponding to the period of greatest thermal stabilization of the cave.

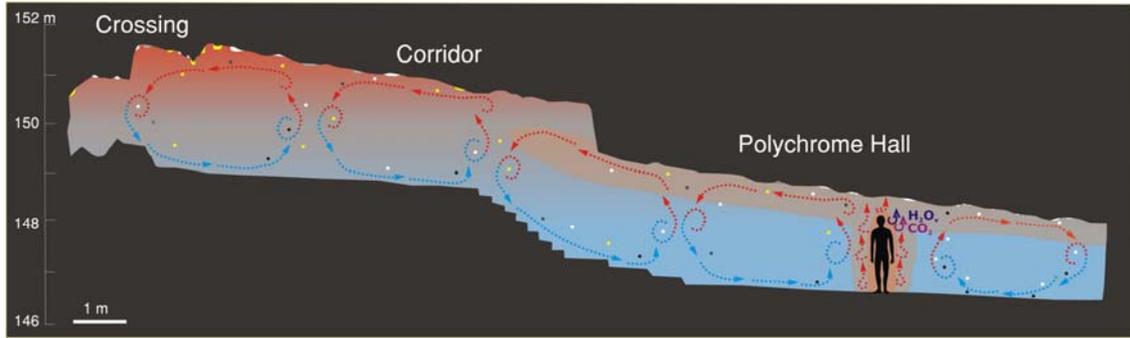


Fig. S6
Ideal scheme of the human disturbances in Polychrome Hall.

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