

Research article

Spatiotemporal distribution of $\delta^{13}\text{C}$ – CO_2 in a shallow cave and its potential use as indicator of anthropic pressure

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ABSTRACT

This study deals with the spatiotemporal dynamics of CO_2 and its isotopic composition ($\delta^{13}\text{C}$ – CO_2) in the atmosphere of Altamira Cave (northern Spain) over two annual cycles. In general terms, the cavity shows two distinct ventilation modes, acting as a CO_2 reservoir from October to May (recharge stage), while actively exchanging gases with the outside atmosphere between July and September (discharge stage). In recharge mode, the in-cave air shows higher and relatively homogeneous CO_2 values (3332 ± 521 ppm) with lower $\delta^{13}\text{C}$ – CO_2 ($-23.2 \pm 0.4\text{‰}$). In contrast, during the discharge stage, the CO_2 concentrations are lower and relatively more variable (1383 ± 435 ppm) and accompanied by higher $\delta^{13}\text{C}$ – CO_2 (up to -12‰). This seasonal pattern is controlled by the distinct rates of exchange of air masses with the external atmosphere through the annual cycle, as well as by changes in the production of CO_2 in the soil and natural fluctuations in the concentration of dissolved inorganic carbon transported by drip water into the cave. In contrast to the interpretations of previous studies in Altamira Cave, no local air intakes into the deepest cave sections were flagged by our $\delta^{13}\text{C}$ measurements. This finding is also supported by analyses of CO_2 and ^{222}Rn in air, density of airborne particles and air temperature. In addition, preliminary experiments examining the visitor-produced disturbances on $\delta^{13}\text{C}$ – CO_2 were conducted during the various cave ventilation stages to explore the potential use of this parameter as an indicator of anthropic pressure in caves. Our data show that visits (overall stay of 60–85 min; i.e., 4 people for 20 min) significantly affected $\delta^{13}\text{C}$ – CO_2 (up to $\Delta\delta^{13}\text{C} \sim -2\text{‰}$) in the Polychrome Hall of Altamira Cave under conditions of low natural CO_2 (discharge stage), whereas it remained almost unaltered under circumstances of high CO_2 concentration (recharge stage). This demonstrates that $\delta^{13}\text{C}$ – CO_2 is sensitive to perturbations produced by visitors during certain periods.

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1. Introduction

CO_2 concentration, along with the temperature and relative humidity of air, has been widely studied in subterranean atmospheres (Pulido-Bosch et al., 1997; Calaforra et al., 2003; Fernández-Cortés et al., 2006; among others). Recently, the isotopic composition of carbon ($^{13}\text{C}/^{12}\text{C}$) in CO_2 , commonly denoted as $\delta^{13}\text{C}$ (its normalized value with respect to the international standard V-PDB, $\delta^{13}\text{C} = 0\text{‰}$), has started to be utilized as a tracer of natural processes occurring in karst terrains (Lambert and Aharon, 2011). Thereby, the isotopic composition of CO_2 in subterranean

atmospheres ($\delta^{13}\text{C}$ – CO_2) has emerged as a new geochemical tool that is usually aimed at identifying the sources of CO_2 and the seasonal patterns that control its spatiotemporal distribution in caves (Spötl et al., 2005; Matthey et al., 2010; Riechelmann et al., 2011; Mandić et al., 2013; among others).

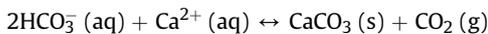
Under natural conditions, CO_2 in cave atmospheres can derive from multiple sources, including soil respiration, exchange of air masses with the external atmosphere, host rock dissolution and contributions from deep-hypogenic CO_2 (Lambert and Aharon, 2011). In general terms, decay of organic matter in the soil, along with plant root and microbial respiration are the main sources of CO_2 to epigeal subterranean environments. CO_2 diffuses into the soil water generating H_2CO_3 with a consequent lowering of pH, which then produces limestone/dolostone karstification.

Bicarbonate (HCO_3^-) is the predominant species of dissolved

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inorganic carbon (DIC) in karstic water, which is usually neutral or slightly basic (pH 7.5–7.8; Ford and Williams, 2007). Once the seepage water reaches a karstic cavity, CO₂ degassing of the solution can occur as long as the pCO₂ of the subterranean atmosphere is lower than that of the water, following the reversible reaction:



This mechanism is also responsible for precipitation of carbonate speleothems in caves (Fairchild et al., 2006).

On a global scale, the $\delta^{13}\text{C}$ of tropospheric CO₂ has a distinctive mean of around -8‰ (Keeling, 1961; Verburg, 2007) and so it can be easily distinguished from edaphic CO₂, which has a more negative $\delta^{13}\text{C}$ of between -26‰ and -12.5‰ , depending on the predominant vegetation cover in the cave setting (i.e., C₃-type vegetation vs C₄-type vegetation). Meanwhile, carbonate bedrock dissolution resulting from high pCO₂ in the soil water is a relevant mechanism of karstification at the contact between the edaphic cover and the regolith beneath, as well as in the epikarst. This process is described by the reversible reaction:



Although the stoichiometry of this reaction would indicate equivalent contributions from soil and host rock dissolution to the DIC of drip water, investigations of radiocarbon mass-balance suggest that inorganic carbon derived from the dissolution of ancient carbonate typically represents less than 10–20% of the total inorganic carbon species dissolved in infiltration water (Genty and Massault, 1997). This is a consequence of the slow kinetics of this reaction, which does not generally reach completion in open systems. Considering that marine carbonate typically exhibits $\delta^{13}\text{C}$ values ranging from 0 to 4‰ (Morse and MacKenzie, 1990), bedrock dissolution could contribute by slightly increasing the $\delta^{13}\text{C}$ of the CO₂ that reaches the cave atmosphere.

The characteristic signatures of $\delta^{13}\text{C}$ -CO₂ in the above-mentioned CO₂ sources enable the origin and dynamics of CO₂ in caves to be traced. In the present paper, we study the spatiotemporal distribution of CO₂ concentration and $\delta^{13}\text{C}$ -CO₂ in Altamira Cave (Cantabria, N Spain) over two annual cycles (2013–2014 and 2014–2015). The goals of this survey were (1) to identify the origin of the CO₂ in the subterranean atmosphere of Altamira Cave and (2) to characterize the seasonal pattern controlling the spatiotemporal changes in $\delta^{13}\text{C}$ -CO₂ and their connection with seasonal variations of $\delta^{13}\text{C}_{\text{DIC}}$ in drip water. Special attention was paid to (3) detecting any “hidden” local air intakes (other than the main cave entrance) as alluded to in previous investigations (García-Antón et al., 2013). This conclusion was based on measurements of CO₂ and CH₄ concentrations in air and $\delta^{13}\text{C}$ -CO₂, in addition to monitoring density of colony forming units (CFU) of bacteria and fungi. In a single sampling survey conducted in July 2012, it was found that CO₂ and CH₄ concentrations were lower in the deepest cave levels and that $\delta^{13}\text{C}$ -CO₂ and CFU were higher than in other nearby areas. This fact is worth noting, since the cited cave sector is more than 150 m apart from the main cave entrance. These authors claimed that during the period of maximum cave ventilation (summer) localized air inputs from the external atmosphere occur in the innermost parts of the cave, producing anomalies in microclimate with respect to nearby cave areas.

In our study, in addition to monitoring CO₂ concentration and $\delta^{13}\text{C}$ -CO₂, we included other microclimate parameters, such as air temperature, ²²²Rn and density of airborne particles, which are also sensitive to the presence of punctual external air intakes. Periodical samplings have been performed over a period of two and a half years, with special focus on the microclimate of the deepest cave

levels during the summer of 2013, 2014 and 2015. The confirmation or refutation of these microclimatic patterns in the deepest cave levels is of extreme importance for planning the future management of Altamira Cave, since continuous inputs of airborne particles and microorganisms could pose a serious threat to the preservation of its prehistoric rock-art heritage (Gázquez et al., 2016). Finally, (4) preliminary experiments addressed to evaluate the impact of visitors on $\delta^{13}\text{C}$ -CO₂ were conducted within the current regime of restricted visits to the renowned “Polychrome Hall” of Altamira Cave. These experiments explored the use of $\delta^{13}\text{C}$ -CO₂ as a potential tool for future management of Altamira Cave and other caverns. This new methodology can be of wide application to study the impacts of visitors in other show caves worldwide.

2. Setting and cave description

Altamira Cave (43°22′37″N; 4°07′11″W) is located near the village of Santillana del Mar (Cantabria, northern Spain), at an elevation of 152 m a.s.l, 4.5 km from the Cantabrian Sea. This cave hosts priceless rock-art paintings, some 36,160–15,329 cal. BP that have earned it the name “Sistine Chapel” of the Palaeolithic, due to the reddish and ochre-coloured bison and deer paintings that cover the ceiling of the renowned “Polychrome Hall” (Gázquez et al., 2016). Altamira Cave was declared a World Heritage Site by UNESCO in 1985 (<http://whc.unesco.org/en/list/310>).

The cavern runs NW-SE and has developed along a sub-horizontal sequence of Cenomanian-Turonian marine carbonates (Hoyos et al., 1981) where the difference in level between the entrance and its deepest part (Grave Hall) is barely 26 m. Several cave sections can be distinguished according to their speleological morphology and distance/depth from the entrance: the Entrance Hall is a 20 m-long horizontal hall; from there, access to the rest of the cave is via an artificial door located in a narrow passage, some 2 m wide. Beyond, the cave divides along two branches. To the east is the “Polychrome Hall”, some 15 m long, 7 m wide and up to 3 m high and lying about 2 m lower than the cave entrance. The other branch gives access to Walls Hall, roughly 50 m in length, 20 m wide and up to 7 m high; located some 6 m below the entrance.

A passage, roughly 60 m long runs SE-NE into the Great Hall. From here, artificial stairs carved in the rock lead to Grave Hall, lying about 15 m below the cave entrance and containing an ephemeral water pool that just appears during periods of high dripwater discharge. To the South, along a 10 m-long passage, lies the “Shower Hall”, which is one of the larger caverns, roughly 40 m long and 20 m wide. This in turn leads into the “Well Hall”, at the depth of 12 m beneath the cave entrance. This cave section accommodates a 5 m-deep pit, though its connection with deeper karstic levels is too small for a person to access. However, previous studies suggested that this shaft has important implications for the Altamira Cave microclimate (García-Antón et al., 2013). Finally, there is a meandriform sub-horizontal passage (called the “Horsetail”) approximately 40 m long that represents the innermost cave area. The thickness of the cap rock overlaying the cave varies from 2 to 18 m (Elez et al., 2013) (Fig. 1). There is no permanent running watercourse inside the cave.

Altamira Cave has a relatively stable microclimate, with air temperatures that vary less than 2 °C throughout the year in the innermost areas (Well Hall and Horsetail) and up to 5 °C in the outermost part of the cave (Entrance Hall) (Quindos et al., 1987; Cuezva, 2008). The annual variation of inside air temperatures exhibits a sinusoidal pattern with a thermal lag time (~2 months on average) between the innermost and outermost cave areas that depends on the outside temperature (Cuezva et al., 2009). The amplitude of the seasonal oscillations differs in the various sections of the cave and is correlated with distance from the cave entrance

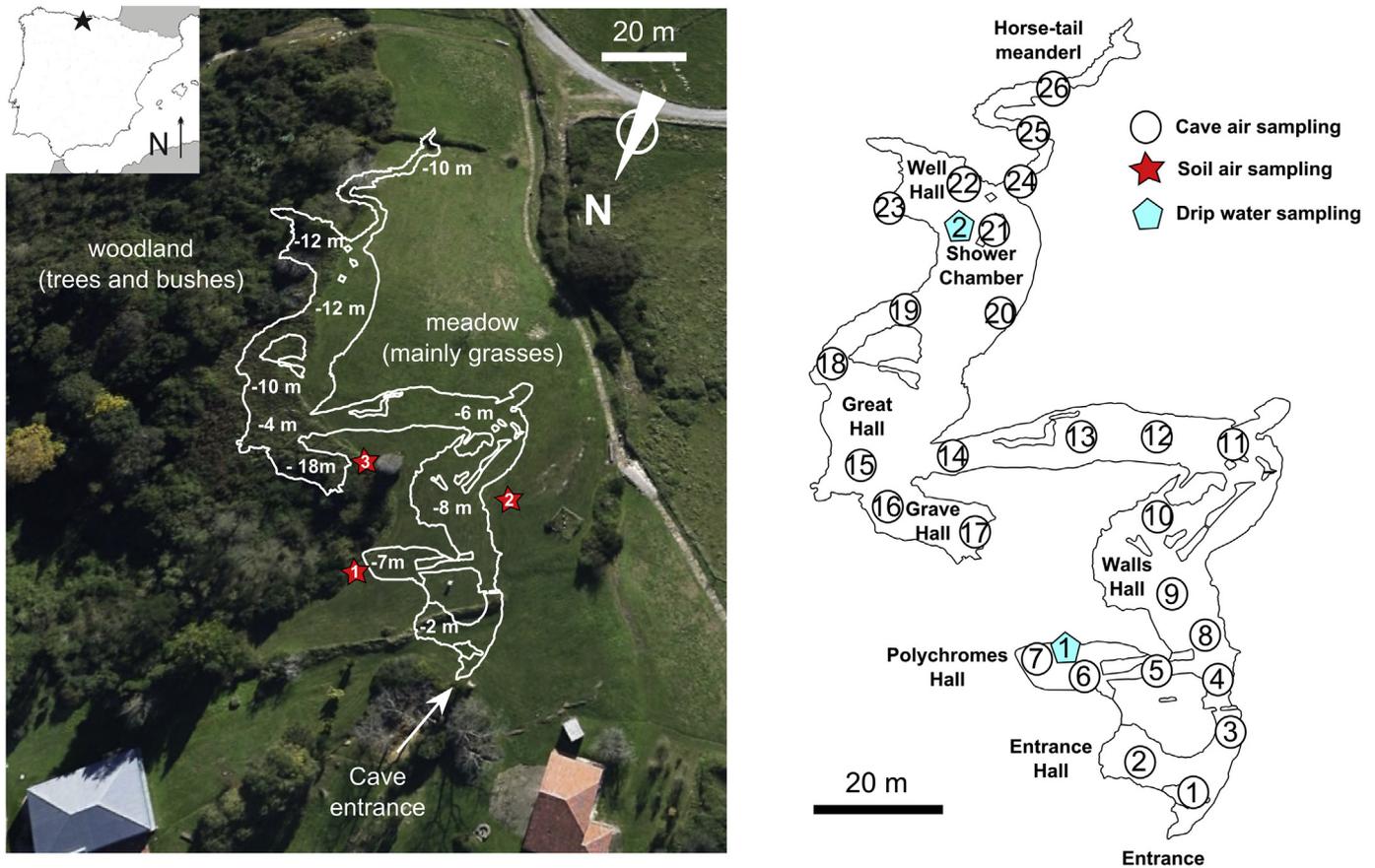


Fig. 1. Altamira Cave: topography and location of the sampling stations (white circle: CO₂ concentration and $\delta^{13}\text{C}-\text{CO}_2$, air temperature, ^{222}Rn concentration and airborne particle density; blue pentagon: dripwater $\delta^{13}\text{C}_{\text{DIC}}$; red stars: CO₂ concentration and $\delta^{13}\text{C}-\text{CO}_2$ in the soil overlying the cave). The thickness of the cap rock over the cave (after Elez et al., 2013) is shown on the topography. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and the thickness of the overlying host-rock. The mean annual temperature outside over the study period was 15.5 °C and the annual relative humidity was 85%, with values above 90% during most of the year.

3. Sampling and analytical methods

3.1. Air and water sampling

Septum-capped 12 mL soda borosilicate vials (Labco®) were utilized for air sampling. First, each vial was open for a couple of minutes at the sampling site, then flushed *in situ* by injecting around 50 mL of cave air, and closed again using a double wadded cap. Subsequently, the vial was manually evacuated by means of a 25 mL syringe provided with a 5 cm needle. The vial was again opened to the cave atmosphere, then evacuated again during 30 s using the syringe. Afterwards, ~15 mL of cave air were injected into the vial. A total of 261 air samples were collected for $\delta^{13}\text{C}-\text{CO}_2$ analysis in 26 cave locations with a periodicity of roughly two months between March 2013 and August 2015. Remarkably, García-Antón et al. (2014) found a variance of up to 300 ppm in the CO₂ concentration and over 1‰ in $\delta^{13}\text{C}-\text{CO}_2$ during a daily cycle in the atmosphere of the Polychrome Hall in September, 2011, with the lower CO₂ recorded during the night (0:00) and the highest levels at 11:00 a.m. This suggests that for proper comparison between the results obtained in different sampling campaigns, air samples must be taken at the same time of the day; this avoids variability due to the natural daily cycle of the cave atmosphere, especially during the

periods of higher ventilation and lower CO₂ concentrations (summer). In all our surveys, air samples were collected between 12:00 p.m. and 14:00 p.m.

Soil air sampling was conducted by means of three bespoke edaphic chambers built from 20 cm tall LDPE jars (~750 cm³). The chambers are provided with multiple 0.5 mm orifices in the lower part, enabling air exchange with the soil atmosphere, and a double wadded cap with a rubber septum on the upper end that allows sampling using a syringe. The first soil air samples were taken in March 2013, one week after the installation of the chambers, and sampling was repeated with the same periodicity as the surveys inside the cave. A total of 27 soil air samples were collected and analysed for CO₂ concentration and $\delta^{13}\text{C}-\text{CO}_2$ over the study period. In addition, two samples of the external air were collected in each survey, 26 samples in all.

The effect of the air exhaled by visitors on CO₂ concentration and $\delta^{13}\text{C}-\text{CO}_2$ in the atmosphere of the Polychrome Hall was studied by collecting air samples at stations #6 and #7 (see Fig. 1) before and after experimental visits. These consisted of a group of two people staying in the hall for 45 min on the 12nd of May, 2014; a group of four people, whose visit lasted for 20 min on the 14th of July, 2014; and a group of three people who visited the Polychrome Hall for 20 min on the 5th of August, 2015. A sample of the air exhaled by each participant was collected using the same 25 mL syringes and subsequently injected in evacuated capped 12 mL vials.

Water samples for $\delta^{13}\text{C}_{\text{DIC}}$ analysis were stored in the same 12 mL vials, poisoned with a drop of saturated solution of ZnCl₂.

Water samples were collected in Polychrome Hall and Shower Hall along with the air samples approximately every two months, making a total of 22 samples (Fig. 1).

In the surveys of July 2013, July 2014 and August 2015, the concentration of CO₂ in air was analysed *in situ* at the same sampling stations as the air samples were collected (Fig. 1), using a Testo 445 (mod. 0560–4450) device with a precision of 5%. Air temperature was measured using mercury thermometers (± 0.1 °C) installed permanently in the cave at stations #2, #3, #4, #5, #7, #9, #13, #17 and #22 (Fig. 1) and measured at the other stations using a digital temperature probe (Vaisala HMP155) sensor with a precision of ± 0.1 °C. The differences between temperature measurements using these two methods at the same cave sites did not exceed 0.2 °C. Airborne particle content (0.01–1 µm) was determined at all sampling stations using a portable CPC (Condensation Particle Counter) probe (TSI 3007) with a precision of $\pm 10\%$ (1SD). ²²²Rn concentration in air was analysed at stations #2, #5, #7, #13, #17 and #22 using passive detectors CR-39 (Radosys), consisting of 100 mm² chips in RSKS type chambers. Each detector was exposed for a period of 15 days inside the cave. The reading of the tracks and the calculation of track density was done automatically with a Radometer 2000, employing routine or slightly modified counting programs for small round tracks, provided by the manufacturer. This method enables “integrated” measurements of ²²²Rn exposure (Bq m⁻³ h) in air, which are then converted to Bq/m³ using the exposure period in hours. In this study, we use ²²²Rn concentration of an interval roughly 15 days long before the $\delta^{13}\text{C}$ –CO₂ samplings of July 2013, July 2014 and August 2015.

3.2. Analytical methods

Air and water samples were analysed at the Stable Isotope Facility of Davis University (CA, USA) within 15–30 days after sampling. $\delta^{13}\text{C}$ analyses in gaseous carbon dioxide were conducted using a ThermoScientific PreCon-GasBench system interfaced with a ThermoScientific Delta V Plus isotope ratio mass spectrometer (ThermoScientific, Bremen, DE). The gas is introduced in a six-port rotary valve (Valco, Houston TX) with a 100 µL loop programmed to switch at the maximum CO₂ concentration in the helium carrier gas. The CO₂ was then separated from other gases by a Poroplot Q GC column (25 m × 0.32 mm ID, 45 °C, 2.5 mL/min). A pure reference gas (CO₂) was used to calculate provisional delta values of the sample peak. Final $\delta^{13}\text{C}$ values were obtained after adjusting the provisional values, such that correct $\delta^{13}\text{C}$ values for laboratory standards were obtained. Isotopic values are reported according to the V-PDB (Vienna Pee Dee Belemnite) scale. Three laboratory internal standards (OZ-3, OZ-10, OZ-40, with $\delta^{13}\text{C}$ values ranging

from –3.63‰ to –40.86‰) were analysed every 10 samples. The laboratory standards are calibrated directly against the NIST 8545 standard. The intensity of the CO₂ signal of the samples was calibrated against the known CO₂ concentration values of the internal standards. The method reproducibility (1SD) was better than 0.1‰, on the basis of the repeated analysis (n = 65) of the OZ-40 internal standard.

For the analyses of $\delta^{13}\text{C}$ in dissolved inorganic carbon (DIC), water samples (1–4 mL) were injected into evacuated 12 mL septum capped vials (Labco®) containing 1 mL 85% phosphoric acid, which forces the equilibrium between CO₂ and H₂CO₃ to gaseous CO₂. The evolved CO₂ is purged from vials through a double-needle sampler into a helium carrier stream (20 mL/min). The gas is introduced into a six-port rotary valve (Valco, Houston TX) with a 100 µL loop programmed to switch at the maximum CO₂ concentration in the helium carrier. The CO₂ is passed to the IRMS through a Poroplot Q GC column (25 m × 0.32 mm ID, 45 °C, 2.5 mL/min). Isotopic values are also reported according to the V-PDB (Vienna Pee Dee Belemnite) scale. Final DIC content and $\delta^{13}\text{C}$ values are obtained after adjusting the provisional values, such that correct DIC and $\delta^{13}\text{C}$ values for laboratory standards are obtained. Two laboratory standards were analysed at intervals of every 10 samples. The laboratory internal standards were a solution of lithium carbonate in degassed deionized water and a deep seawater reference material (both calibrated against NIST 8545). The method reproducibility (1SD) was better than 0.1‰, from the repeated analysis (n = 23) of the lithium carbonate solution.

3.3. Spatial representation of data

The spatial maps of cave air temperature were constructed using geostatistical gridding based on an ordinary point kriging method, with a linear variogram model using Surfer 8® software. During interpolation and calculation of the empirical semivariogram, a preferential direction was considered, running along the main cave axis (NE-SW) for characterizing the spatial variability of the environmental parameters (CO₂ concentration, $\delta^{13}\text{C}$ –CO₂, air temperature, ²²²Rn content in air and density of airborne particles).

4. Results

4.1. Spatiotemporal distribution of CO₂ and $\delta^{13}\text{C}$ –CO₂

The mean CO₂ concentration in the soil chambers above Altamira Cave during the study period was 1936 ± 1297 ppm and its $\delta^{13}\text{C}$ was $-20.0 \pm 1.9\%$. Chamber 1 exhibited the lowest CO₂ concentrations (1226 ± 616 ppm) and more enriched isotopic values

Table 1
 $\delta^{13}\text{C}$ of CO₂ in soil over Altamira Cave (see Fig. 1 for location) between March 2013 and May 2015.

Month/year	Edaphic chamber 1		Edaphic chamber 2		Edaphic chamber 3	
	$\delta^{13}\text{C}$ (‰)	CO ₂ (ppm)	$\delta^{13}\text{C}$ (‰)	CO ₂ (ppm)	$\delta^{13}\text{C}$ (‰)	CO ₂ (ppm)
Mar-13	–18.5	1661	–21.8	2790	–22.7	6227
May-13	–21.2	2223	–22.5	1874	–22.2	4513
Jul-13	–17.4	606	–19.6	1270	–	–
Sep-13	–17.0	687	–	–	–19.5	1152
Nov-13	–17.1	831	–	–	–20.2	1239
Jan-14	–	–	–18.5	1108	–20.6	1480
Mar-14	–17.2	946	–19.4	1625	–19.9	1446
May-14	–20.6	1629	–20.7	1843	–22.2	2768
Jul-14	–	–	–21.7	2760	–23.0	3436
Jan-15	–	–	–18.6	924	–16.9	884
May-15	–	–	–	–	–20.7	2489
AVG	–18.4	1226	–20.3	1775	–20.8	2563
1SD	1.8	613	1.5	703	1.8	1733

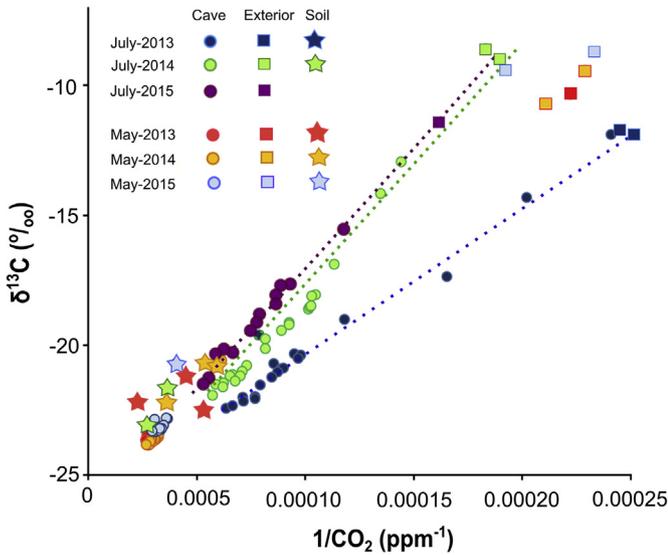


Fig. 2. Keeling plot ($\delta^{13}\text{C}$ vs $1/\text{CO}_2$) of CO_2 during discharge (e.g., July) and recharge (e.g., May) stages in Altamira Cave in different surveys.

($-18.4 \pm 1.8\%$). The mean $\delta^{13}\text{C}\text{-CO}_2$ values in chambers 2 and 3 were similar (-20.3 ± 1.5 and -20.8 ± 1.8 , respectively); however, the former gave significantly lower CO_2 concentrations (1775 ± 703 ppm) than the latter (2436 ± 1733 ppm) over the duration of this survey (Table 1). No significant seasonal trends were observed either in CO_2 or $\delta^{13}\text{C}$ in our study. As expected and according to the Keeling (1958) model, $\delta^{13}\text{C}$ is positively correlated with $1/\text{CO}_2$ ($R^2 = 0.81$), which indicates that the higher soil CO_2

concentrations coincide with isotopically depleted carbon (Fig. 2).

García-Antón et al. (2013) found similar CO_2 and $\delta^{13}\text{C}\text{-CO}_2$ values in the soil above Altamira Cave during a three-day point study conducted in July 2012 (1085 ± 140 ppm and -20.6% , for CO_2 and $\delta^{13}\text{C}$ respectively). In contrast, García-Antón et al. (2014) reported considerably higher values for CO_2 (8047 ± 4575 ppm), isotopically lighter (-26.5%) in another three-day survey performed in September 2011.

The CO_2 concentration in the outside atmosphere showed a mean of 497 ± 78 ppm, whereas its $\delta^{13}\text{C}$ was $-10.5 \pm 1.5\%$ on average, with no identifiable seasonal oscillation. These values agree with the measurements of $\delta^{13}\text{C}\text{-CO}_2$ performed in previous studies at the same location (García-Antón et al., 2013, 2014).

The CO_2 measurements obtained *in situ* in the cave are closely correlated ($R^2 = 0.98$) with the results from the spectrometric analysis in the laboratory (CO_2 concentration and $\delta^{13}\text{C}\text{-CO}_2$). In this

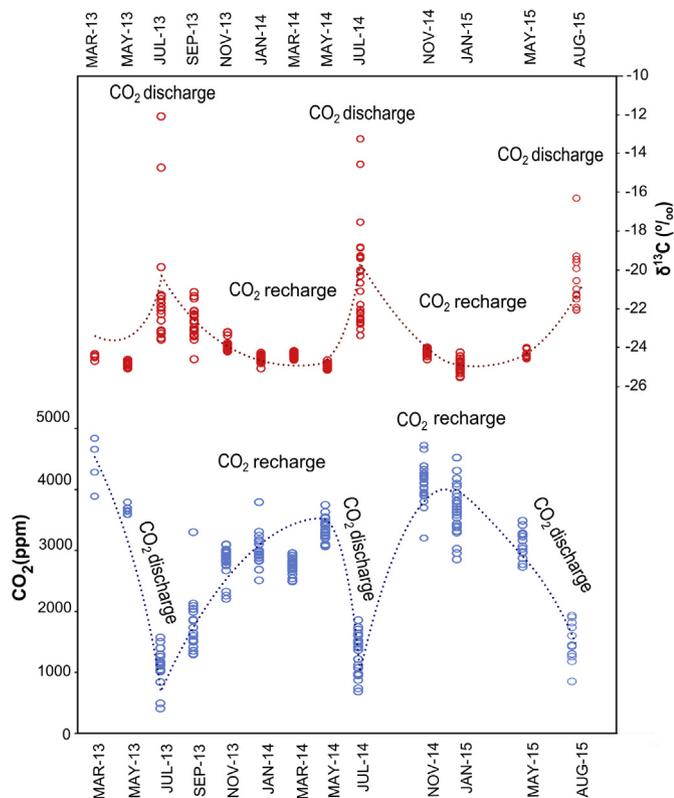


Fig. 3. CO_2 concentration and $\delta^{13}\text{C}\text{-CO}_2$ in the Altamira Cave atmosphere over the duration of this study.

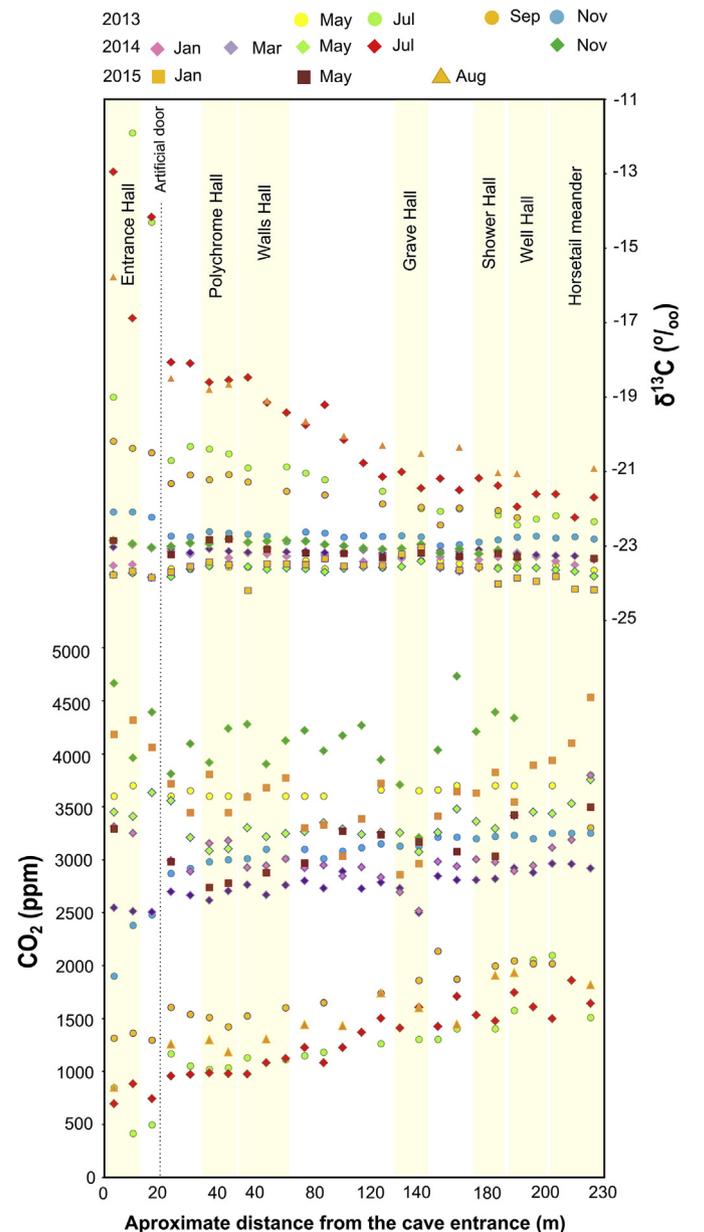


Fig. 4. Spatial distribution of CO_2 concentration and $\delta^{13}\text{C}\text{-CO}_2$ in Altamira in 12 sampling surveys conducted between 2013 and 2015.

study, we report the results from the spectrometric measurements. Over the duration of the survey, the range of CO₂ concentration in the subterranean atmosphere of Altamira Cave was 2774 ± 1008 ppm, whereas its $\delta^{13}\text{C}$ was $-22.4 \pm 1.8\text{‰}$. CO₂ concentration showed noticeable temporal trends throughout the annual cycle, which was reproduced at all cave stations (Fig. 3). CO₂ exceeded 4500 ppm in March 2013, November 2014 and January 2015. Generally, CO₂ values were higher between November and May in both annual cycles studied, whilst the CO₂ concentration was lower than 1000 ppm at some cave points in July 2013, July 2014 and August 2015. Likewise, $\delta^{13}\text{C}$ -CO₂ presented clear seasonal oscillations, showing greater depletion between November and May (e.g., -23.8‰ in May 2013 or -24.2‰ in January 2015) and more enriched carbon isotopes in summer (e.g., up to -12‰ in July 2013 in the upper cave levels). As also observed in the soil samples, $\delta^{13}\text{C}$ is positively correlated with $1/\text{CO}_2$ across the complete data set (Fig. 2).

In terms of spatial distribution, $\delta^{13}\text{C}$ -CO₂ showed a very different pattern in summer (when CO₂ concentration is lower) compared with the rest of the annual cycle (when CO₂

concentration is higher). Both CO₂ and $\delta^{13}\text{C}$ values were more homogenous during autumn-winter-spring (e.g., ± 136 ppm and $\pm 0.1\text{‰}$ in March 2014, 1SD), whereas samples collected from the innermost part of the cave (Horsetail) and the Entrance Hall produced indistinguishable values (within the error of the CO₂ and $\delta^{13}\text{C}$ analyses) in all surveys. In contrast, relatively more disperse CO₂ and $\delta^{13}\text{C}$ values were observed in summer (e.g., ± 410 ppm and $\pm 2.7\text{‰}$ in July 2013, 1SD) along the cave profile when compared with the autumn-winter-spring measurements. For instance, the atmosphere in the outmost cave area (Entrance Hall) in July 2013 had CO₂ concentrations with $\delta^{13}\text{C}$ values (415 ppm and -11.9‰), similar to samples of the external atmosphere taken close to the cave entrance (409 ppm and -11.7‰) (Fig. 3). This indicates active air homogenization in the outermost cave areas with respect to the outside atmosphere. In contrast, these two points differed by up to 4200 ppm CO₂ and 13‰ $\delta^{13}\text{C}$ in winter (i.e., January 2015), showing complete lack of connection between the external atmosphere and the in-cave air during this period.

Unlike under the autumn-winter-spring regime, noticeable trends in CO₂ and $\delta^{13}\text{C}$ -CO₂ with depth can be distinguished in

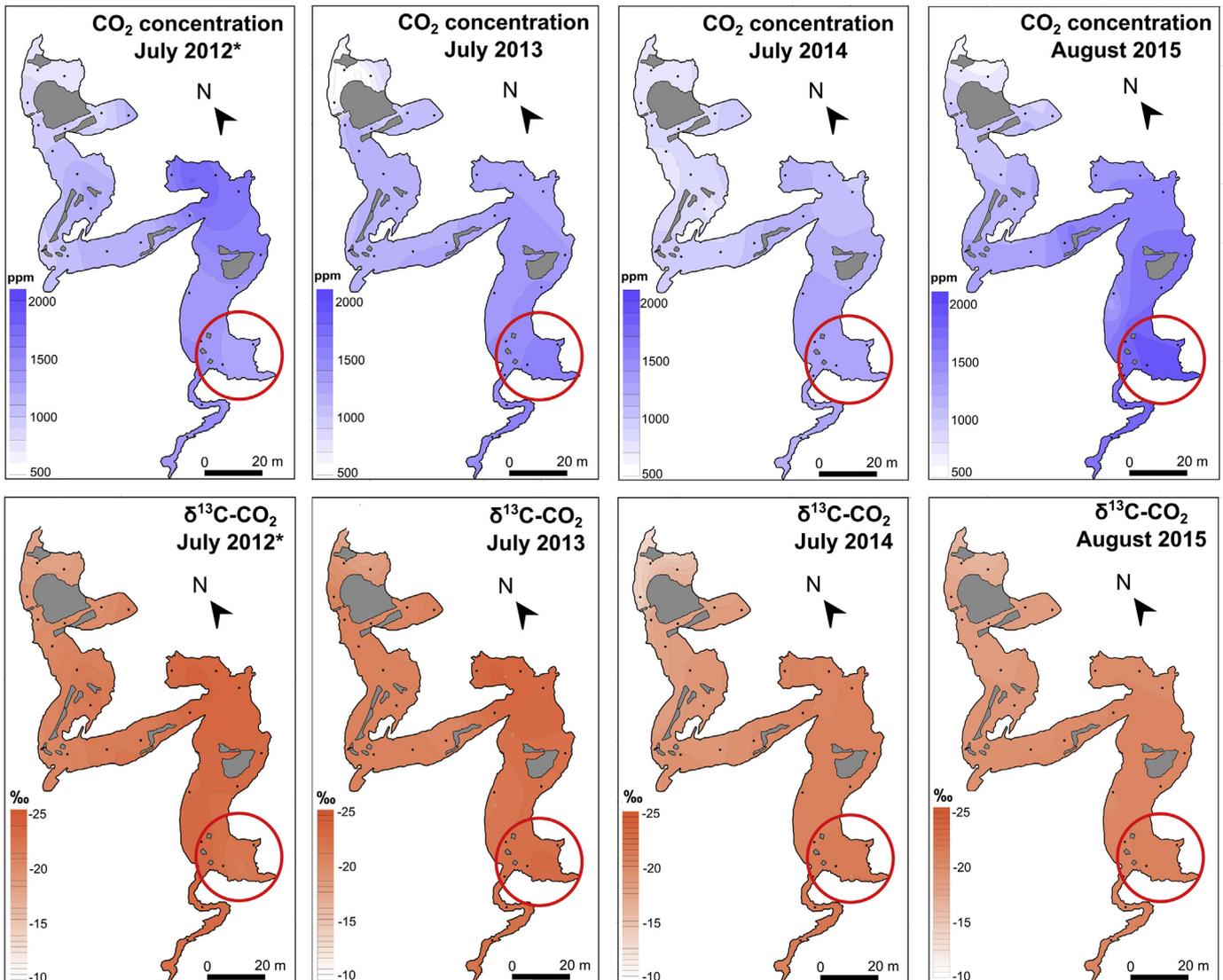


Fig. 5. Spatial distribution of CO₂ concentration and its $\delta^{13}\text{C}$ in Altamira Cave in July 2012 (García-Antón et al., 2013)*, July 2013, July 2014 and August 2015 (this study). Note that the small anomalies in CO₂ concentration and $\delta^{13}\text{C}$ observed in Well Hall (red circles) in 2012 were not repeated in 2013, 2014 or 2015. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2

CO₂ concentration and δ¹³C–CO₂ in the air of Altamira Cave at sampling stations in Shower Hall, Well Hall and in the HorseTail meander (Fig. 1). We compare our results (July 2013, July 2014 and August 2015) with the data reported by García-Antón et al. (2013)* of July 2012.

	Shower hall (#21)		Well hall 1 (#22)		Well hall 2 (#23)		Horse tail 1 (#24)	
	CO ₂ (ppm)	δ ¹³ C (‰)	CO ₂ (ppm)	δ ¹³ C (‰)	CO ₂ (ppm)	δ ¹³ C (‰)	CO ₂ (ppm)	δ ¹³ C (‰)
23-Jul-12*	1374	–22.4	1720	–23.3	–	–	1564	–22.7
26-Jul-12*	1356	–23.3	1195	–21.8	–	–	1492	–23.7
15-Jul-13	1402	–22.2	1578	–22.4	2052	–22.3	2095	–22.2
14-Jul-14	1478	–21.4	1745	–21.9	1611	–21.6	1498	–21.6
5-Aug-15	1907	–21.1	1934	–21.1	–	–	1818	–20.9

summer (Fig. 5). CO₂ increases toward the deepest and innermost cave areas, while δ¹³C is depleted (Fig. 4). The differences between the Entrance Hall and the deeper passages were up to ~1500 ppm CO₂ and 11‰ in δ¹³C–CO₂ in July 2013. The differences decreased to ~100 ppm in CO₂ and roughly 1‰ in δ¹³C–CO₂ in November 2013. Remarkably, the artificial door that separates the Entrance Hall from the rest of the cave has a significant effect on CO₂ concentration and δ¹³C during summer (Fig. 3), whereas no measurable influence has been observed at other times of year. As mentioned

above, practically no differences in CO₂ concentration and δ¹³C were observed through the rest of the annual cycle in the various sections of the cave.

Special attention was focussed on the Well Hall section, where previous studies had claimed to have detected a presumed “hidden” air inflow. In July 2012, the air in this cave location contained slightly lower CO₂ values (313 ppm less) and was more enriched in carbon isotopes (up to 1.9‰) than nearby cave areas (Shower Hall and Horsetail) (García-Antón et al., 2013) (Fig. 5). Our

Table 3

Effect of air exhaled by visitors in the atmosphere of the Polychrome Hall of Altamira Cave. The CO₂ concentration and its δ¹³C in the air exhaled by the visitors were also analysed.

Experiment #1										
12 nd May 2014	Start time:	12:10	End time:	12:55	Visitors:	2	δ ¹³ C	CO ₂		
SAMPLING STATION	δ ¹³ C _{before}	δ ¹³ C _{after}	Δ ¹³ C	CO ₂ _{before}	CO ₂ _{after}	ΔCO ₂	Visitor #1	-25.9	12841	
Polychrome (#6)	1	-23.5	-23.7	-0.2	3084	3270	+186	Visitor #2	-25.2	20195
Polychrome (#7)	2	-23.5	-23.6	-0.1	3104	3291	+187	AVG	-25.6	16518
							1SD	0.5	5199	
Experiment #2										
14 th July 2014	Start time:	12:40	End time:	13:00	Visitors:	4	δ ¹³ C	CO ₂		
SAMPLING STATION	δ ¹³ C _{before}	δ ¹³ C _{after}	Δ ¹³ C	CO ₂ _{before}	CO ₂ _{after}	ΔCO ₂	Visitor #1	-24.5	17668	
Polychrome (#6)	1	-18.6	-21.3	-2.7	987	1072	+85	Visitor #2	-24.9	17154
Polychrome (#7)	2	-18.5	-20.2	-1.7	979	1223	+244	Visitor #3	-23.5	34069
							Visitor #4	-24.2	6770	
							AVG	-23.8	20420	
							1SD	0.6	11281	
Experiment #3										
5 th August 2015	Start time:	12:30	End time:	12:50	Visitors:	3	δ ¹³ C	CO ₂		
SAMPLING STATION	δ ¹³ C _{before}	δ ¹³ C _{after}	Δ ¹³ C	CO ₂ _{before}	CO ₂ _{after}	ΔCO ₂	Visitor #1	-27.0	28950	
Polychrome (#6)	1	-18.8	-19.2	-0.4	1300	1378	+79	Visitor #2	-24.1	10049
Polychrome (#7)	2	-18.7	-19.6	-0.9	1185	1368	+184	Visitor #3	-25.2	26267
							AVG	-25.2	25267	
							1SD	1.5	10226	

measurements of the same variables at the same cave locations showed that the atmosphere in the Well Hall section (#22) in July 2013 contained slightly higher CO₂ than the nearby Shower Hall (#21) and lower values than the first sampling station in the Horsetail (#24). In July 2014, the sample collected at this sampling site also contained higher CO₂ than nearby sampling stations. As for δ¹³C, no differences within the analytical error were found at the different air sampling stations in this cave section in July 2013, whereas it was 0.5‰ lower in Well Hall than in Shower Hall in July 2014 (Table 2). This pattern repeated in August 2015, with practically no differences in δ¹³C–CO₂ or CO₂ concentration between these cave locations (Table 2 and Fig. 5).

4.2. Effect of air exhaled by visitors on δ¹³C–CO₂

The first experimental visit to the Polychrome Hall of Altamira Cave consisted of two people for a period of 40 min in May 2014, when the CO₂ in the in-cave air is highest (Fig. 3). This visit resulted in an increase of up to 187 ppm CO₂ and a practically insignificant lowering in δ¹³C–CO₂ by 0.2‰. During the experiment conducted in July 2014, when CO₂ concentrations in the cave are at a minimum, the presence of 4 visitors over 20 min produced a CO₂ increase of up to 244 ppm and a significant decrease in the δ¹³C–CO₂ value of –2.7‰. The air exhaled by the visitors was significantly enriched in CO₂ and its δ¹³C–CO₂ value was considerably more negative than the natural in-cave atmosphere. The same phenomenon was observed in August 2015, when three visitors stayed in the cave for 20 min, increasing CO₂ by up to 184 ppm and depleting the δ¹³C–CO₂ value by almost 1‰ (Table 3).

4.3. Concentration of dissolved inorganic carbon (DIC) and δ¹³C_{DIC} in dripwater

The DIC concentration in dripwater ranged from 25.1 mg/L to 50.6 mg/L over the period of this study. Lower concentrations were typically found in samples collected during summer (July–September), whereas samples taken in winter-spring contained higher concentrations at the two locations studied. δ¹³C_{DIC} was inversely correlated with DIC concentration across the whole data set, showing enrichments as high as –11.1‰, in September 2013 in the Polychrome Hall. In contrast, the depleted isotopic values – below –15‰, correspond to samples collected in winter-spring (Table 4).

Table 4
Concentration of dissolved inorganic carbon (DIC) and δ¹³C_{DIC} in dripwater at the two sampling stations in Polychrome Hall and Shower Hall of Altamira Cave (see Fig. 1).

	Polychrome hall		Shower hall	
	DIC (mg/L)	δ ¹³ C (‰)	DIC (mg/L)	δ ¹³ C (‰)
Mar-13	47.5	–14.4	42.2	–14.2
May-13	43.5	–14.5	38.0	–14.7
Jul-13	28.5	–13.3	43.3	–13.9
Sep-13	27.6	–11.1	25.1	–12.9
Nov-13	40.5	–12.9	33.7	–13.5
Jan-14	49.5	–14.5	43.4	–14.5
Mar-14	44.4	–13.9	42.7	–14.9
May-14	44.9	–14.6	47.1	–14.7
Jul-14	36.9	–12.7	41.3	–15.1
Nov-14	49.6	–12.1	50.6	–15.0
Jan-14	36.9	–15.3	39.4	–15.3
AVG	40.9	–13.6	40.6	–14.4
1SD	7.7	1.3	6.8	0.7

4.4. Air temperature, ²²²Rn concentration and airborne particle density

The in-cave air temperature during the surveys of July 2013, July 2014 and August 2015 varied from 13.2 to 20.6 °C (Fig. 6). In all surveys, air temperature decreased from the cave mouth to the deepest/innermost cave locations. The artificial door that separates the Entrance Hall from the rest of the cave has a significant effect on the air temperature distribution, similar to the pattern observed for CO₂ and δ¹³C–CO₂. For example, there was a difference of 2.4 °C between samples taken on either side of the door in July 2014. We did not detect any “thermal anomaly” in air temperature in Well Hall compared to the nearby sampling stations in July 2013, July 2014 or August 2015. Grave Hall – the lowest part of the cave – registered lower air temperatures than the rest of the cave in all three surveys (~13.3 °C) (Fig. 6).

The ²²²Rn content in air ranged from 620 to 1687 Bq/m³ in July 2013, from 560 Bq/m³ to 1956 Bq/m³ in July 2014 and from 554 Bq/m³ to 1532 Bq/m³ in August 2015. In all three surveys, ²²²Rn increased with depth (Fig. 6). The highest ²²²Rn values were recorded in Grave Hall and the Horsetail, the deepest and innermost parts of the cave, respectively. Like CO₂ concentration, δ¹³C–CO₂ and air temperature, ²²²Rn did not show any negative anomalies in Well Hall with respect to nearby sampling stations in any of the surveys (Fig. 6).

Lastly, the airborne particle content varied from 180 to 1814 particles/cm³ in July 2013, from 480 to 3716 particles/cm³ in July 2014 and from 356 to 3112 particles/cm³ in August 2015; it decreased with depth and distance from the cave entrance, with higher concentrations close to the entrance and the lowest in the deepest and inmost cave areas. Like the other parameters, airborne particles in Well Hall did not show significant differences with respect to the other locations in the inner cave sections in July 2013, nor in July 2014 or August 2015 (Fig. 6).

5. Discussion

5.1. Origin and spatiotemporal distribution of CO₂ in Altamira Cave

It is broadly accepted that there is a close relationship between the type of vegetation cover and the isotopic composition of the CO₂ supplied from respiration of plants and microorganisms to the edaphic atmosphere. C₃-type plants, whose metabolism follows the Calvin cycle – representing over 98% of plant species – typically produce tissue- and root-derived CO₂ with isotopic values around –26‰. On the other hand, C₄-type plants, mainly grasses and steppe species, have less discrimination towards ¹³C, derived from the Hatch-Slack metabolic cycle, and their biomass is typically enriched, with values around –12.5‰ for δ¹³C (O’Leary, 1981).

Our results of δ¹³C–CO₂ from the soil overlying Altamira Cave (–20.0 ± 1.9‰; Table 1) indicate that C₃-type plants are responsible for most of the edaphic CO₂. Both CO₂ concentration and δ¹³C–CO₂ were quite variable during the period study but did not show any significant correlation with the main annual climatic pattern. Very probably, these values do not exactly reflect primary CO₂ production of soil but are the result of a mixture of edaphic and atmospheric CO₂ in varying proportions depending on punctual climatic and biological parameters, including soil temperature and saturation, atmospheric pressure and wind speed, etc. In other words, the CO₂ concentration in the shallower soil horizons is controlled by short-term climatic and biological processes and exchange of gases with the atmosphere, which in some cases results in low CO₂ concentrations and δ¹³C–CO₂ closer to the values of the external atmosphere (884 ppm and –16.9‰, in Jan 2015).

The Keeling plot in Fig. 2 shows the relationship between CO₂

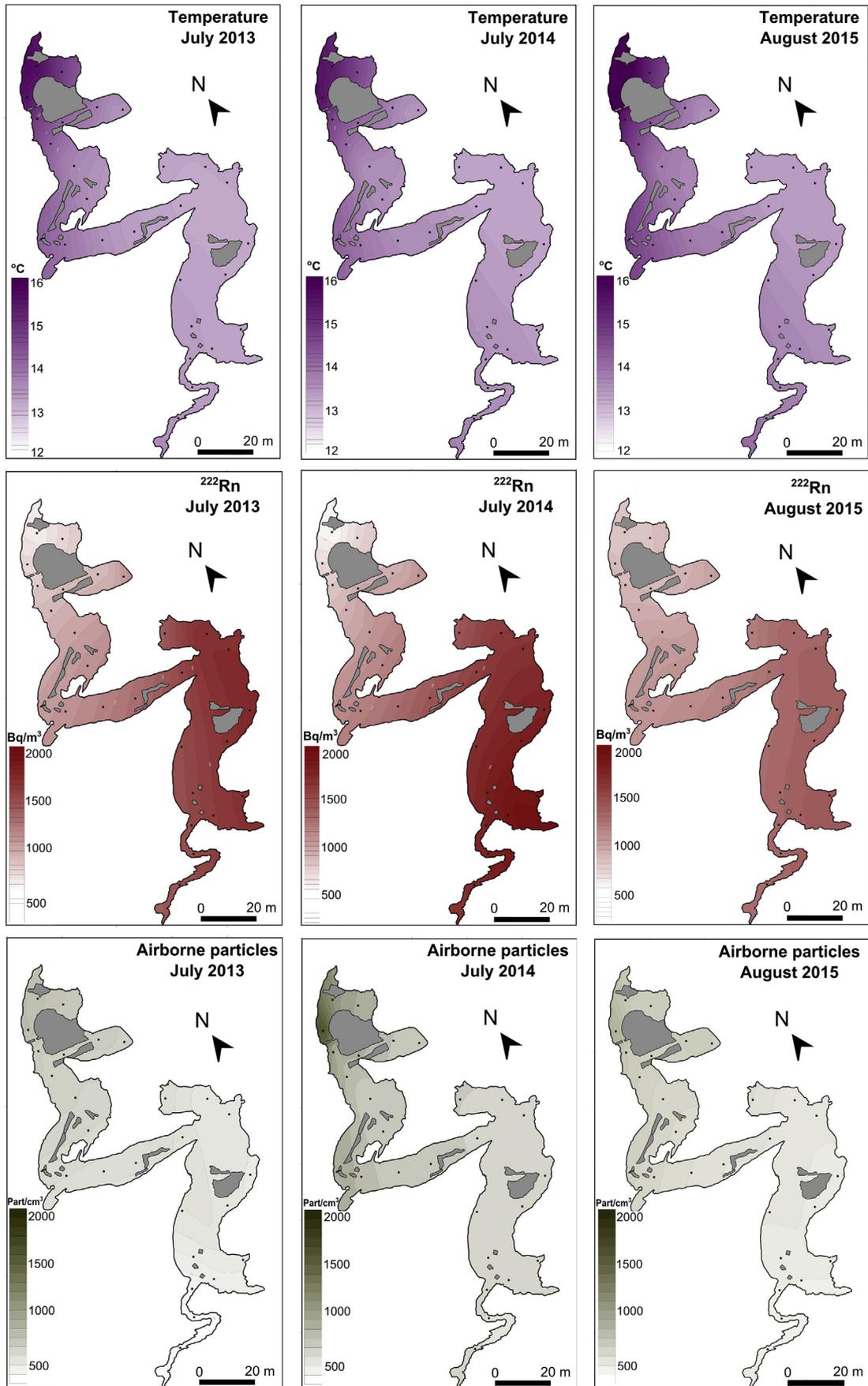


Fig. 6. Spatial distribution of air temperature, ^{222}Rn concentration and airborne particle density in air in Altamira Cave in July 2013, July 2014 and August 2015.

concentrations and $\delta^{13}\text{C}-\text{CO}_2$ in the in-cave air, the soil and the external atmosphere during May and July. In this two-endmember plot, the cut with the Y-axis represents the actual $\delta^{13}\text{C}-\text{CO}_2$ value of the soil endmember, roughly -26‰ , which agrees well with previous studies in this cave ($\sim 28\text{‰}$; García-Antón et al., 2013). The difference between our value and the mean isotopic value of the cave atmosphere ($-22.5 \pm 1.7\text{‰}$) broadly agrees the expected values due to the isotopic fractionation that occurs along the path of CO_2 from the soil to the cave atmosphere. This includes the isotopic enrichment of $8\text{--}10\text{‰}$ that takes place between the gaseous CO_2 and HCO_3^- in the soil water, which varies slightly according to soil temperature and pH (e.g., Mook and de Vries, 2000; Scholz et al., 2009). This suggests that the $\delta^{13}\text{C}_{\text{DIC}}$ in the soilwater may be -15 to -17‰ . Accordingly, we found that $\delta^{13}\text{C}_{\text{DIC}}$ in dripwater matches these values, especially during autumn-winter-spring ($\sim -15\text{‰}$ in January–March; Table 4), whereas it increases considerably in the summer ($\sim -12\text{‰}$ in July–September; Table 4). Enriched $\delta^{13}\text{C}_{\text{DIC}}$ values during the drier months could be a consequence of enhanced CO_2 -degassing of the solution and prior calcite precipitation in karstic conduits (Baker et al., 1997).

Alternatively, intense CO_2 -degassing when the solution reaches the cave ceiling could also explain such offset, mainly in the discharge stage, when the $p\text{CO}_2$ in the cave atmosphere is reduced and CO_2 -degassing is favoured. During degassing, the CO_2 released to the atmosphere is isotopically lighter by around 8‰ for $\delta^{13}\text{C}$ (Mattey et al., 2010), which explains the values of around -22‰ in the Altamira Cave atmosphere during the autumn-winter-spring, as well as in the innermost cave areas during the summer (Fig. 5). Although dripwater degassing has been proposed as producing important CO_2 contribution to caves, especially in the case of deep caves (e.g., Baker et al., 1997), direct gaseous diffusion between the subterranean and external atmosphere through fractures in the bedrock and the edaphic cover cannot be ruled out; this was also suggested in previous studies in Altamira Cave (Cuezva et al., 2011; García-Antón et al., 2014), given that the carbonate caprock does not exceed 3 m in thickness over some parts of the cave.

During summer (discharge stage, hereafter), the in-cave air shows lower CO_2 concentrations and higher $\delta^{13}\text{C}-\text{CO}_2$ that suggest intense air exchange with the external atmosphere (Fig. 3). Additional evidence of the connection between the outside CO_2 and the in-cave atmosphere is given by the different slopes of the $\delta^{13}\text{C}-1/\text{CO}_2$ lines in July 2013 and July 2014/2015 with respect to the atmospheric endmember, whose value changed slightly over the time of this study. This empirical relationship indicates that the in-cave values lie on a line where the $\delta^{13}\text{C}$ -depleted endmember matches the CO_2 concentrations and $\delta^{13}\text{C}-\text{CO}_2$ of the external atmosphere on the day of the sampling, so demonstrating that intense ventilation and air renewal occurs during the discharge stage in Altamira Cave, especially in the outermost cave sectors but also in the deeper areas (Fig. 2). This air renewal in Altamira Cave is controlled by differences in the air temperature and density between the in-cave and the external atmosphere, described in detail by Cuezva et al. (2011) and García-Antón et al. (2014). We hypothesize that CO_2 emitted from the cave can also derive from diurnal changes in CO_2 concentration and $\delta^{13}\text{C}-\text{CO}_2$ in the vicinity of the cave, especially during the discharge period.

Over the remainder of the year, CO_2 concentration increased from September to May (recharge stage hereafter), producing the highest values between November and March. Simultaneously, $\delta^{13}\text{C}-\text{CO}_2$ decreased at all sampling stations over the recharge stage and its variability was less than during the discharge stage in the various cave areas (Figs. 3 and 4). This indicates that during the recharge stage, CO_2 is gradually stored in the cave due to increased CO_2 inputs from the soil caused by higher infiltration during the wetter period and reduced air exchange with the exterior.

In regard to the spatial distribution of the CO_2 concentration and $\delta^{13}\text{C}-\text{CO}_2$ in the cave, our data show that during the discharge stage there are significant differences between the shallower, outermost areas (e.g., Entrance Hall) and the deeper, inner sectors (e.g., Grave Hall and Horsetail) (Figs. 4 and 5). The CO_2 concentration decreases almost linearly with distance from the cave entrance as $\delta^{13}\text{C}-\text{CO}_2$ decreases. During this cave phase, the artificial door that separates the Entrance Hall from the rest of the cave plays a role by dramatically reducing air mass exchange with the external atmosphere, as indicated by the difference of up to 1000 ppm in CO_2 concentration and 9‰ $\delta^{13}\text{C}-\text{CO}_2$ observed between these two nearby areas in the July surveys. In contrast, during the discharge stage, the dispersion of the CO_2 concentration and $\delta^{13}\text{C}-\text{CO}_2$ between the different cave sections is less, with practically no alteration due to the artificial door. This demonstrates that during this cave phase, air mass movements are drastically reduced.

5.2. Investigating the presence of potential “hidden” air inflows

Given the relatively high CO_2 concentrations with depleted $\delta^{13}\text{C}$ values in the atmosphere of Altamira Cave, any air intake from the external atmosphere (CO_2 around 497 ± 78 ppm and $\delta^{13}\text{C}$ of $-10.3 \pm 1.3\text{‰}$) would produce identifiable changes in the in-cave atmosphere. As explained in the previous section, during the discharge stage of the cave, air from the external atmosphere replaces the in-cave air mass stored during the recharge stage, producing a decrease in CO_2 concentration and lower $\delta^{13}\text{C}$ values in the cave. The presence of “hidden” local air inflows (other than the main cave entrance) would cause point anomalies in all the parameters studied compared to nearby cave areas.

García-Antón et al. (2013) suggested the presence of a localized air intake to the cave in Well Hall, on the basis of measurements of CO_2 and CH_4 , $\delta^{13}\text{C}-\text{CO}_2$ in air and CFU (colony-forming units) analyses. In a single sampling survey conducted in July 2012, these authors found that CO_2 and CH_4 concentrations were lower in this cave section and that $\delta^{13}\text{C}-\text{CO}_2$ and CFU were higher than in other nearby areas, such as the Horsetail meander and Shower Hall (Table 2 and Fig. 5). As stated by these authors, this “hidden” inflow could have implications for air mass dynamics in the cave and ingress of bacterial and fungal spores, with important consequences for the conservation of the cave paintings in Altamira Cave.

In order to verify or reject this crucial point, to the CO_2 and $\delta^{13}\text{C}-\text{CO}_2$ measurements, we performed *in situ* analyses of air temperature, ^{222}Rn concentration and airborne particle density at the same sampling stations as during the surveys conducted in July 2013, July 2014 and August 2015. In contrast to the previous result, we did not detect any evidence of air inflow in the Well Hall area. In all three surveys, this section showed slightly higher CO_2 concentrations with lower $\delta^{13}\text{C}$ than at the nearest sampling site in Shower Hall (Table 2 and Fig. 5). This is contrary to what would be expected if there was an inflow of air at this site. Likewise, our air temperature measurements did not show any anomalous values in Well Hall compared to Shower Hall and Horsetail, whereas any air flux from the external atmosphere ($\sim 20^\circ\text{C}$) would produce a differential temperature increase at this cave location (Fig. 6).

The results of the ^{222}Rn measurements and airborne particle density in air were similar. ^{222}Rn is a radioactive gas that is naturally produced from the decay of uranium and other radioactive atoms in the carbonate host-rock in caves. ^{222}Rn is accumulated in the subterranean atmosphere and usually covaries with CO_2 concentration (e.g., Gregorić et al., 2013). Unlike CO_2 , the ^{222}Rn source to the cave atmosphere is constant over time and so its variations are exclusively related to cave ventilation. For this reason, ^{222}Rn concentration in caves has been widely used to infer air mass dynamics in a variety of caves (e.g., Kowalczyk and Froelich, 2010). The

presence of diffuse or localized air inflows produces air mass renewal and reduces the ^{222}Rn concentration in air. Such ^{222}Rn dilution was not detected in Well Hall of Altamira Cave during the discharge stage surveys, which is when – according to García-Antón et al. (2013) – the presumed air intake occurs (Fig. 6). Likewise, a direct connection between the cave and the exterior would result in a higher density of airborne particles, since in the external atmosphere these are much higher than in the in-cave air (up to 5200 particles/cm³ measured in July 2014). Again, anomalous values of this parameter were been observed in Well Hall during the discharge stage (Fig. 6).

In summary, using our multianalytical approach we did not detect any evidence of “hidden” air inflows in Well Hall of Altamira Cave, as previously claimed by García-Antón et al. (2013). Nevertheless, assuming that their measurements are right, we suggest that this circumstance could occasionally occur in some areas of Altamira Cave, depending on particular meteorological conditions in the exterior (*i.e.*, abrupt atmospheric pressure changes). Such mechanism of diffuse or point air mass exchange through alternative entrances is common in karstic terrain, in which the heterogeneous distribution of pores and conduits in the carbonate host rock is a fundamental aspect to be considered (Ford and Williams, 2007).

5.3. Potential usage of $\delta^{13}\text{C}\text{--CO}_2$ as indicator of anthropic pressure in caves

CO_2 concentration has been widely applied to evaluate the effects of visitors on cave atmospheres over the past 20 years (Pulido-Bosch et al., 1997; Faimon et al., 2006; Lang et al., 2015; among others). In our study, we determined the impact of experimental groups of visitors on CO_2 concentration and its $\delta^{13}\text{C}$ in the Polychrome Hall of Altamira Cave.

The experiments were conducted under different climatic modes of the cave – discharge and recharge stages – during which the cave atmosphere contains less and more CO_2 , respectively. We performed three experiments in which the duration of the visit (*i.e.*, the sum of the duration of every participant) ranged from 60 to 85 min. In the experiment conducted in May 2014 (2 visitors, 45 min each) the initial CO_2 concentration was around 3100 ppm and the visit produced an increase of 180 ppm in CO_2 concentration, while $\delta^{13}\text{C}\text{--CO}_2$ was barely affected (less than -0.2‰). In contrast, in a similar experiment in July 2014, when the initial CO_2 concentration was around 980 ppm, the mean ΔCO_2 was also around 180 ppm but $\delta^{13}\text{C}\text{--CO}_2$ sharply diminished (by 2.2‰ on average, Table 3). A similar response was observed in August 2015 (3 visitors, 20 min), when the average change was 120 ppm, with a depletion of 0.7‰ in $\delta^{13}\text{C}\text{--CO}_2$. This indicates that for a similar increase in the CO_2 concentration produced by the visitors' respiration, $\delta^{13}\text{C}\text{--CO}_2$ is more significantly affected in the discharge stage than the recharge phase. In July, the initial $\delta^{13}\text{C}\text{--CO}_2$ was clearly enriched as a result of more intense air mass circulation in the cave and mixing with the outside atmosphere. The impact of the isotopically depleted CO_2 (-23.8‰) exhaled by the visitors in this cave microclimate was higher than during the May experiment, when there was more CO_2 in the cave atmosphere and its $\delta^{13}\text{C}\text{--CO}_2$ more negative, similar to that exhaled by the visitors.

6. Concluding remarks

The isotopic composition of CO_2 ($\delta^{13}\text{C}$) in the atmosphere of Altamira Cave was studied in detail over 30 months, along with the main sources of CO_2 (soil atmosphere, external air and inorganic carbon species transported by dripwater). The in-cave air shows higher CO_2 with $\delta^{13}\text{C}$ values similar to the edaphic atmosphere

between October and May, as a result of reduced air exchange with the outside atmosphere and CO_2 accumulation in the cave over this period. The opposite situation is observed in summer (July–September), when CO_2 is at its lowest and there is greater enrichment in $\delta^{13}\text{C}$, especially in the outer cave areas as a consequence of intense air mass exchange with the external environment. No air intakes other than the main cave entrance were detected by our *in situ* measurements (air temperature, ^{222}Rn content and density of airborne particles). However, the occurrence of local air fluxes into the cave cannot be ruled out, given the thinness of the soil and caprock overlying the cave at some points, and the potential for karstic connections with other cavities in the vicinity of Altamira Cave.

Importantly, the experiments evaluated the impact of visitors on the $\delta^{13}\text{C}\text{--CO}_2$ in the Polychrome Hall of Altamira Cave and showed significant changes in this parameter caused by the CO_2 exhaled by visitors, which has a distinctive isotopic signature. The degree of disturbance on the microclimate was dependent on the natural CO_2 concentration and $\delta^{13}\text{C}\text{--CO}_2$ of the in-cave air. This disturbance was higher under summer (discharge stage) conditions.

Our results demonstrate that $\delta^{13}\text{C}\text{--CO}_2$ in the atmosphere of certain caves can be utilized together with other traditional parameters (*e.g.*, air temperature, relative humidity) to evaluate tourist impact on subterranean environments as a new management tool, and in particular in Altamira Cave. To validate this possibility, it would be necessary to study the variation of these parameters over a greater number of visits, comparable to each other both in terms of duration and number of visitors.

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