

Methane in underground air in Gibraltar karst

Mattey, D.p.; Fisher, R.; Atkinson, T.c.; Latin, J.-p.; Durrell, R.; Ainsworth, M.; Lowry, D.; Fairchild, Ian

DOI:

[10.1016/j.epsl.2013.05.011](https://doi.org/10.1016/j.epsl.2013.05.011)

License:

Creative Commons: Attribution (CC BY)

Document Version

Publisher's PDF, also known as Version of record

Citation for published version (Harvard):

Mattey, DP, Fisher, R, Atkinson, TC, Latin, J, Durrell, R, Ainsworth, M, Lowry, D & Fairchild, I 2013, 'Methane in underground air in Gibraltar karst', *Earth and Planetary Science Letters*, vol. 374, pp. 71-80.
<https://doi.org/10.1016/j.epsl.2013.05.011>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

Eligibility for repository : checked 3/11/2014

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.



Methane in underground air in Gibraltar karst



D.P. Matthey^{a,*}, R. Fisher^a, T.C. Atkinson^b, J.-P. Latin^c, R. Durrell^c, M. Ainsworth^c,
D. Lowry^a, I.J. Fairchild^d

^a Department of Earth Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK

^b Department of Earth Sciences, University College London, London WC1E 6BT, UK

^c Gibraltar Cave Science Unit, Gibraltar Ornithological and Natural History Society, Jews Gate, Gibraltar

^d School of Geography, Earth & Environmental Sciences, University of Birmingham, Birmingham B15 2TT, UK

ARTICLE INFO

Article history:

Received 25 January 2013

Received in revised form

3 May 2013

Accepted 6 May 2013

Editor: G. Henderson

Available online 13 June 2013

Keywords:

cave
methane
carbon isotopes
ground air
ventilation
methanotrophy

ABSTRACT

Little is known about the abundance and geochemical behaviour of gaseous methane in the unsaturated zone of karst terrains. The concentrations and $\delta^{13}\text{C}$ of methane in background atmosphere, soil air and cave air collected at monthly intervals over a 4 yr period are reported for St. Michaels Cave, Gibraltar, where the regional climate, surface and cave processes are well documented. Methane concentrations measured in Gibraltar soil are lower than the local background atmosphere average of 1868 ppb and fall to < 500 ppb. The abundance– $\delta^{13}\text{C}$ relationships in soil air methane lack strong seasonality and suggest mixing between atmosphere and a ^{12}C depleted residue after methanotrophic oxidation. Methane abundances in cave air are also lower than the local background atmosphere average but show strong seasonality that is related to ventilation-controlled annual cycles shown by CO_2 . Cave air methane abundances are lowest in the CO_2 -rich air that outflows from cave entrances during the winter and show strong inverse relationship between CH_4 abundance and $\delta^{13}\text{C}$ which is diagnostic of methanotrophy within the cave and unsaturated zone. Anomalies in the soil and cave air seasonal patterns characterised by transient elevated CH_4 mixing ratios with $\delta^{13}\text{C}$ values lower than -47% suggests intermittent biogenic input. Dynamically ventilated Gibraltar caves may act as a net sink for atmospheric methane.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Karstification and development of caves in terrestrial carbonates are a consequence of downwards percolation of meteoric water through the CO_2 concentration gradients between the atmosphere, soil zone and bedrock that drive calcite dissolution and precipitation processes (Ford and Williams, 2007). Methane and carbon dioxide are closely linked in the carbon cycle but little is known about the abundance and geochemical behaviour of gaseous methane in the unsaturated zone in karst terrains. Methane solubility in water is around 1% that of the solubility of CO_2 and although concentrations are not often reported, methane is a common trace constituent in groundwater (e.g. Barker and Fritz, 1981). The formation of methane from carbon in organic matter or CO_2 and the consumption of methane are both biologically mediated processes at ambient temperatures in near surface environments. Methane formation is a widespread process as the final step in the decay of organic matter by anaerobic respiration of archaea (e.g. Thauer et al., 1989). Methane consumption (oxidation to CO_2) takes place via microbial action under

both aerobic (utilising oxygen) and anaerobic (utilising sulphate or nitrate) conditions (e.g. Raghoebarsing et al., 2006). Oxidic groundwaters of Mesozoic carbonate aquifers in the UK contain very low levels of dissolved methane (around 10 ppb) as a consequence of the effects of microbial oxidation (Darling and Gooddy, 2006). In locally anoxic environments the oxidation of methane to CO_2 can continue as an anaerobic process by microbial consortia of bacteria and archaea that utilise sulphate or nitrate (Raghoebarsing et al., 2006).

Methane is potentially an interesting tracer within the soil–karst–cave system because of its contrasting behaviour to CO_2 , sensitivity to redox conditions and large carbon isotopic fractionations resulting from biogenic processes. Whilst there are known examples of high concentrations of methane in caves that are related to bat and insect ecosystems (Sarbu et al., 1996) or the action of chemoautotrophic bacteria (Hutchens et al., 2004), where such extreme environments are absent the behaviour of methane at natural background levels in the unsaturated zone of limestone karst is largely unknown.

The passage of water plays a central role in karstic processes but gas exchange between the cave interiors and the outside atmosphere is also an important factor that controls carbonate dissolution and re-precipitation. Cave air is enriched in CO_2 relative to the outside atmosphere (Atkinson, 1977a; Baldini, 2010; Benavente et al., 2010; Bourges et al., 2001; Ek and Gewalt, 1985; Ford and Williams, 2007; Matthey et al., 2010; Wood and Petraitis, 1984) and ventilation with background

* Corresponding author. Tel.: +44 1784 443582.

E-mail addresses: matthey@es.rhul.ac.uk (D.P. Matthey).

atmosphere has the effect of diluting the high CO₂ mixing ratios in cave air. Ventilation of atmosphere into the unsaturated zone can be caused by changes in atmospheric pressure (cave breathing), forced ventilation by wind and flowing water, and by convection and chimney circulation (Fairchild and Baker, 2012). Density driven circulation, caused by the temperature contrast between the cave interior and exterior, through interlinked cave chambers with entrances at different levels can create seasonal reversing ventilation controlling calcite dissolution and precipitation as a result of changing cave air pCO₂ (e.g. Banner et al., 2007; Matthey et al., 2010; Spotl et al., 2005). In highly permeable karstified limestone, density driven circulation also takes place in the bedrock in which the caves are embedded—cf. conduit and diffuse flow components of water circulation in the saturated zone of karst aquifers (Atkinson, 1977b; White, 1969). The steep relief of the karstic limestone Rock of Gibraltar promotes this seasonal, density driven circulation of ground air (the CO₂-rich air reservoir permeating the unsaturated zone (Atkinson, 1977a)).

Methane is a trace component of background atmosphere and typical background maritime air at mid-northern latitudes contains around 1850 ppb CH₄ with a δ¹³C value around −47‰ (Dlugokencky et al., 2009; Lowry et al., 2001; Miller et al., 2002). Atmospheric methane is largely derived from wetlands and anthropogenic emissions and the main sinks are via oxidation in the upper atmosphere and methanotrophs in soils (Roslev et al., 1997). The role of the soil zone for methane in the karst environment is therefore crucially different to that of CO₂ and air that penetrates shallow karst via the soil zone should be depleted in atmospheric-sourced CH₄. Such depletion can be expected also to continue in the bedrock, especially in the epikarst where air circulates slowly via fractures (often filled with soil and organic matter) providing an environment for methanotrophy to continue. Thus methane present in cave air may be from several possible sources: residual methane from the soil zone, atmospheric methane from ventilation through cave entrances (bypassing the soil zone) or locally generated by biogenic processes. Biogenically formed methane is significantly enriched in ¹²C relative to atmosphere whereas methane derived from the soil zone is isotopically depleted in ¹²C as a result of microbial oxidation (Whiticar, 1999). Consequently the isotopic composition of methane in cave air should provide a new means of tracing gas exchange pathways and discriminating biogenic and background sources.

St. Michaels Cave, Gibraltar has been the subject of a comprehensive cave monitoring programme since 2004 to improve understanding of the way that climate proxies preserved in speleothem archives are related to surface weather, and how local processes operating in the soil and cave environment affect the trace element and stable isotope properties of speleothem calcite. Gibraltar is a site where the regional climate, surface and cave microclimates, vadose-zone hydrology and speleothem chemistry are well documented (Matthey et al., 2008, 2010) and in this paper we focus on the methane data obtained for background atmosphere, soil air and cave air collected at monthly intervals over a 4 yr period. The results show that methane behaviour is a useful tracer of background atmosphere and additional biogenic sources in the unsaturated zone, and provide evidence that ventilated karst environments are a net sink for atmospheric methane.

2. Site description, local climate, sampling and analytical methods

2.1. Topography, climate and vegetation

The Rock of Gibraltar (36°8'N 5°21'W) forms a N–S trending ridge 2.5 km long with a maximum elevation of 423 m composed of steeply dipping massive limestones and dolomites of Liassic age (Figs. 1–3)

(Rose and Rosenbaum, 1991). The soil is a brown sandy loam becoming redder at depth and much of the soil cover on the upper rock is rather discontinuous as fissure infill and pockets of soil between rock outcrops. Excavations and road cuts show that many fissures are partially filled with fine grained red soil to a depth of 1 or 2 m, even where there is no fine grained material at the surface. The Gibraltar climate is strongly seasonal both in temperature and precipitation (Matthey et al., 2008, 2010; Wheeler, 2006) and the concentration of rainfall during the cooler months of winter leads to an annual surplus of water that infiltrates into the permeable fractured carbonate bedrock and ultimately recharges the ground water table close to sea level. Daily temperature (measured at the cave entrance 275 m asl), and rainfall (measured at the RAF Met Office Station 3 km north of the cave site (Fig. 1)) for 2004–2009 monitoring period are shown in Fig. 4 and the annual and seasonal rainfall and temperature data for the 2004–2009 monitoring period of our study are compared with the equivalent 1960–2000 averages in Table 1.

The vegetation on the Upper Rock around the soil sampling site directly above the cave (Fig. 2) consists predominantly of olive and pine woodland (*macquis*), shrub-herb woodland (*garrigue*) and areas of open, stony ground with grasses and herbs (Linares et al., 1996). Maximum water availability is in winter. The growing season commences with the onset of seasonal rains in autumn and lasts until late spring and is most vigorous at times when water availability is combined with warmth, i.e. in late autumn or early winter and again in spring. The hot, dry conditions of summer and early autumn cause herbs and understory vegetation to die back and all except trees and very deep-rooted plants are then dormant. Thus production of organic matter and leaf fall are strongly seasonal but rates of CO₂ production by decay of organic matter may be regulated by moisture conditions as well as temperature.

2.2. St. Michael's Cave System

The location and plan of the St. Michaels Cave System are shown in Figs. 1 and 2. There are no streams or resurgences related to St. Michaels Cave and the various entrances have been formed

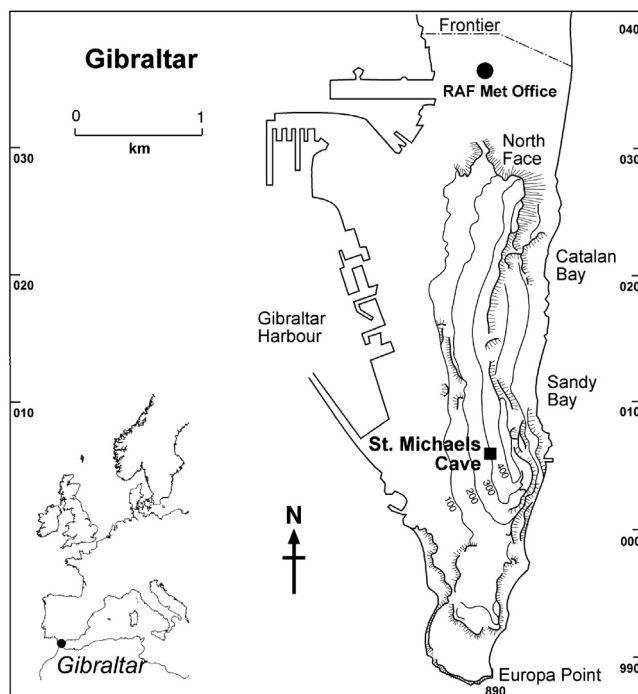


Fig. 1. Map showing the location of Gibraltar, St. Michaels Cave and the meteorological station.

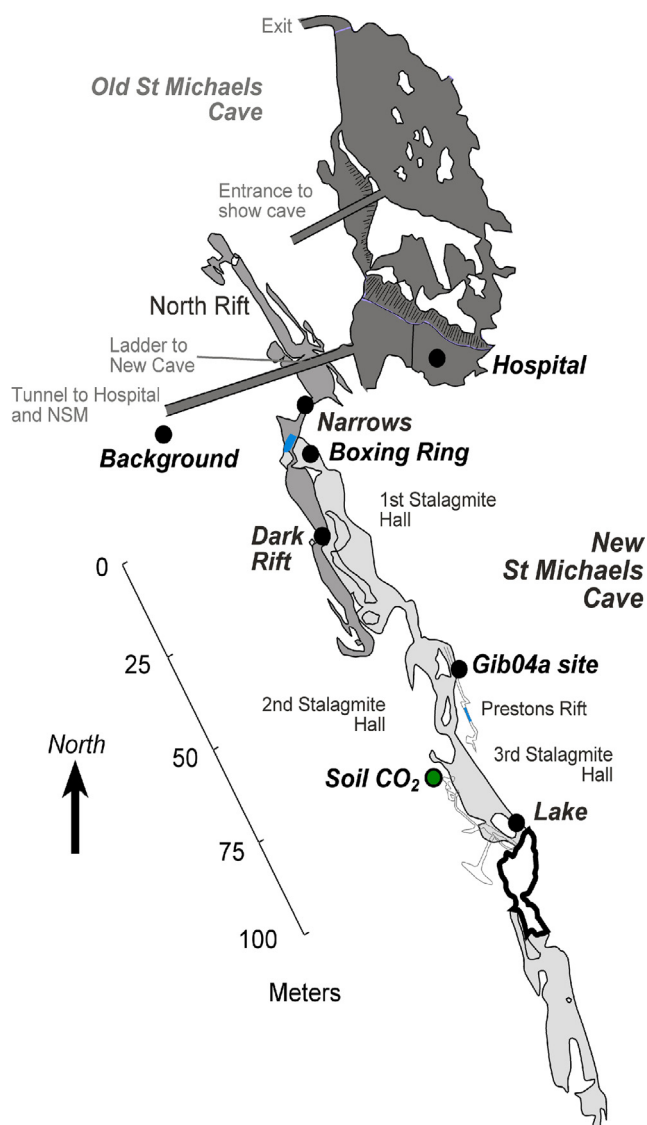


Fig. 2. Plan of the St. Michaels Cave system based on the original cave surveys and diagrams (Rose and Rosenbaum, 1991; Shaw, 1953a, 1953b) showing the location of cave air and soil sampling sites used for this study.

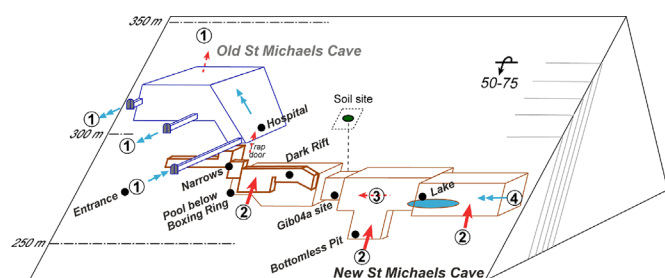


Fig. 3. Schematic isometric block model of the St. Michaels cave system showing the relative positions of entrances, air sampling sites and the position of the soil air sampling site. Bedding of the Gibraltar Limestone and direction of advective transport of air within the cave under winter conditions are also shown: (1) chimney ventilation involving background atmosphere of Old St. Michaels Cave via access tunnels and high level natural entrances; (2) upwards advection of CO_2 -rich ground air; (3) weak lateral air movement through New St. Michaels cave, rising through laddered hatchway into the show cave; (4) weak ingress of background atmosphere from unknown passages leading south that possibly connect via fractures or obstructed fissures to nearby Levant Cave.

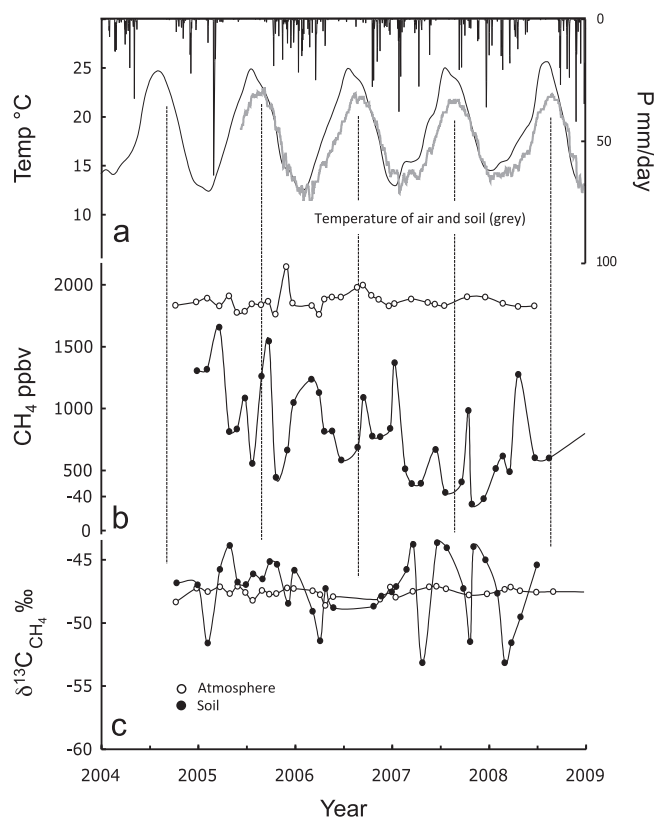


Fig. 4. Variations of local climate and the abundance and carbon isotopic composition of CH_4 in background air and soil air from 2004 to 2008. Year labels mark the start of the calendar year. The upper part of the diagram shows data for daily precipitation amount, mean daily temperature measured at the Gibraltar Met Office and soil temperature measured at 50 cm (grey curve). The variations of CH_4 abundances and $\delta^{13}\text{C}$ values measured in grab samples taken at monthly intervals in soil and background atmosphere are plotted as closed and open circles. Vertical dashed lines mark the timing of highest summer temperatures. Meteorological data Crown Copyright The Met Office, UK.

Table 1

Meteorological statistics for the monitoring period compared to the 1960–2000 average. Winter is defined as the six-month period from October to April; the annual cycle spans winter and is the twelve-month interval from July to June. Data Crown Copyright the UK Meteorological Office.

	2004/5	2005/6	2006/7	2007/8	2008/9	1960–2000
P , mm (winter)	554.1	667.7	629.8	653.6	703.6	
P , mm (annual)	559.6	676.9	690.1	771.9	843.3	763
P -ET (annual)	284.4	428.9	102.7	169.7	276.4	
T , °C (winter)	14.9	15.1	16.0	16.5	14.8	
T , °C (annual)	18.5	18.4	18.7	19.0	18.4	18.3

by erosion or as a result of tunnelling (Rose and Rosenbaum, 1991). Formation of Gibraltar caves which are distributed over a 350 m range in altitude is thought to have taken place as a result of mixing corrosion between groundwater of meteoric origin and sea water permeating the water table underneath the rock (Tratman, 1971). The caves now exist at their present elevated altitudes as a consequence of neotectonic uplift (Rodríguez-Vidal et al., 2004). Old St. Michaels Cave (OSM) (Shaw, 1953b) has developed along the strike of dolomitic limestone creating a large main chamber. Enlargement along bedding planes created minor caves linked to OSM that provide several natural entrances to the system along with two entrances via artificial tunnels (Fig. 3). A lower series of natural passages leads southwards along the strike of the Gibraltar limestone at an altitude of 275 m (Fig. 3) and is known as New

St. Michaels Cave (NSM) (Shaw, 1953a). It is accessed through a laddered hatchway from a tunnel cut in 1942 into the lowest part of OSM (the 'Hospital').

The various entrances from the west-facing hillside into OSM lie between 260 and 303 m altitude and a strong natural chimney ventilation is developed between them (Matthey et al., 2010). Airflows show seasonal reversals in flow direction: in winter, warm cave air flows upwards drawing cool, denser outside air into the lower entrances whereas in summer cave air flows out of lower entrances, drawing warmer, less dense atmosphere into upper entrances.

Within the NSM system, which has only a single known entrance via the Hospital trap door and ladder (Fig. 2) seasonally reversing slow flows of air can be detected using chemical smoke as a tracer. However the dramatic seasonal fluctuations in CO₂ levels described by Matthey et al. (2008, 2010) suggest that these currents are due to an advective exchange of cave air with the permeable networks of fractures and fissures in the surrounding bedrock, which in turn connect to the ground surface. The strongly seasonal regime of CO₂ in cave air reflects this advective transport of ground air through the upper part of the Rock, with NSM acting as a conduit discharging ground air in winter, and in summer accepting external air and distributing it into the surrounding ground air reservoir. The winter air circulation in NSM is illustrated in Fig. 3 and discussed in greater detail below.

2.3. Sampling sites, air collection and analytical methods

2.3.1. Soil and cave air grab sampling

Samples for soil gas analysis were collected from a small area vertically above the chamber containing stalagmite Gib04a (Matthey et al., 2008) (Figs. 2 and 3). Soil air grab samples were taken at monthly intervals by two methods. Before June 2007 the monthly samples were taken through a 6 mm OD steel tube inserted 30–50 cm through undisturbed soil to the bedrock–soil interface at two new positions within a designated area of approximately 2 m². Soil gas was drawn in laterally through angled holes behind the closed tip using a low flow pump to fill 1 L or 3 L Tedlar bags. From June 2007 the sampling was carried out from porous PTFE sampling cups (Ecotech, Germany) buried at 50 cm and 25 cm in an excavated and back-filled pit 15 cm in diameter. Each cup was laid in a layer of gravel 6 cm thick. A conservative calculation based on uniform porosity of 33% and radially symmetrical flow to the point of extraction that the sampled domain would not intersect the surface. The data reported are the mean of the two samples taken by each method.

A temperature logger (Gemini TinyTag2) at 50 cm depth recorded hourly soil temperatures. Samples of the soil itself were taken at 10 cm intervals from the surface to the bedrock during installation of the above equipment, for determination of carbon abundance and isotopic composition of soil organic matter.

Cave and ambient air sampling was carried out using a low flow pump to fill 1 L or 3 L Tedlar bags at a rate of 200 mL/min. Cave air sampling was carried out as the first task in each chamber by pumping through a 5 m tube to avoid contamination by respired CO₂ and bag samples were returned to RHUL and analysed usually within 2 weeks of sampling.

2.3.2. Mixing ratio analysis

For CO₂ and CH₄ mixing ratio analysis air samples were pumped from the Tedlar bags into an evacuated 150 mL sample volume to a pressure of 2.5 bar, using a diaphragm pump (KNF Neuberger). Samples were then analysed for methane using an HP5890 gas chromatograph with flame ionisation detector (GC-FID) or a Picarro cavity ring down spectrometer. Mixing ratios are on the NOAA 04

scale (Dlugokencky et al., 2009). Measurements are made by comparison with an internal secondary standard which was calibrated by measurement against one of four NOAA standards, in the range 1819–2033 ppb. The resultant precision is better than 3 ppb (1 σ) for mixing ratios less than 3000 ppb. Accuracy has not been assessed below 1800 ppb as no methane in air standards were available at below background atmospheric levels and errors may be between 10 and 100 ppb for the very lowest concentrations measured at some points in the cave.

2.3.3. Isotope analysis

Isotope analyses of methane were made using a GV Instruments TraceGas–Isoprime system (Fisher et al., 2006). Samples were analysed directly from the bag connected to a Cajon fitting on the automated inlet section of the Trace Gas. The sample volume on the inlet to the TraceGas was 75 cc for ambient concentrations, but a larger volume, 150 cc, was used for the methane in cave air which had very low concentrations.

On each day of analyses using the Trace Gas, the internal secondary standard tank (RHS 584 or RHS 615) was run regularly, at least four times at the start of the day and between at least every 10 sample analyses. This secondary standard is a tank of air collected at RHUL which has also been analysed for methane $\delta^{13}\text{C}$ using an off-line extraction technique and dual inlet analysis (which has a precision–standard deviation in replicate analyses of $\pm 0.03\text{‰}$) (Lowry et al., 2001). The number of analyses depended on how much sample was available—ideally 3 measurements were made but sometimes there was only enough sample for 1 or 2 measurements. In a few cases there was not enough air remaining for methane isotopic analysis. The precision (1 σ) of methane $\delta^{13}\text{C}$ analysis, based on 10 consecutive analyses of the secondary standard tank, was better than 0.09‰.

3. Results

The results obtained for the 2004–2008 monthly time series are presented in Fig. 4 (atmosphere and soil) and Fig. 5 (cave air); these data along with additional analyses of air samples taken at less regular intervals until 2010 are compiled in Fig. 6.

3.1. CH₄ in background atmosphere

Methane is the main hydrocarbon in the atmosphere with a northern hemisphere clean air average abundance of around 1850 ppb (Lowry et al., 2001). Air samples analysed in Gibraltar are slightly higher than the clean air background containing an average of 1868 ppb with a range of 1760–2148 ppb. A weak correlation between elevated CH₄ and elevated CO₂ suggests that elevated CO₂ and CH₄ values are both derived from local industrial sources. The $\delta^{13}\text{C}$ of background CH₄ averages -47.6‰ with a small range of 1.5‰ and the monthly samples in this study show no clear evidence of seasonality in the abundances and isotopic composition of either CO₂ or CH₄ (Fig. 4b and c).

3.2. CH₄ in soil air

The abundance and isotopic composition of CH₄ in monthly samples of soil air are plotted in Fig. 4b and c. Methane concentrations in Gibraltar soil are lower than in background atmosphere, typically ranging from 1500 ppb to less than 500 ppb as a result of oxidation of atmospheric methane by methanotrophic bacteria residing in soil (Cicerone and Oremland, 1988; Roslev et al., 1997). Seasonal fluctuations are not evident but CH₄ levels steadily decrease year by year from 2005 to 2008, a trend which inversely

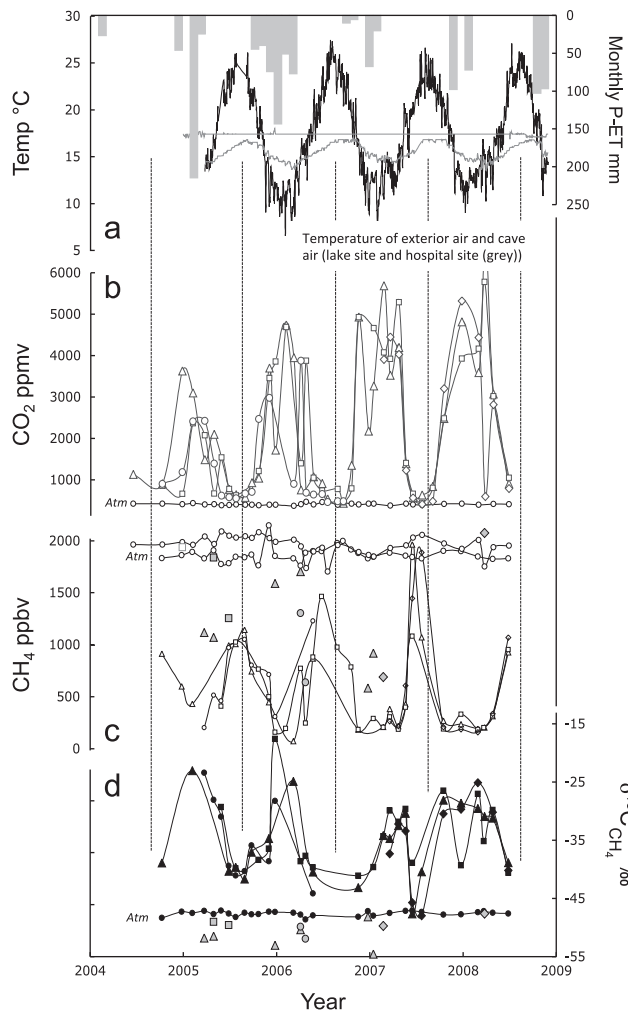


Fig. 5. Variations of cave temperature, water excess and the abundance and carbon isotopic composition of CH_4 in cave air from 2004 to 2008. The upper part of the diagram shows data for monthly water excess expressed as P-ET calculated using the Thornthwaite method (Thornthwaite, 1954) and the mean daily air temperatures measured at the New St. Michaels Cave entrance and cave air temperatures at the Hospital (grey curve) and Lake sites (Fig. 2). Vertical dashed lines mark the timing of highest summer temperatures. The variations in abundance (open symbols) and $\delta^{13}\text{C}$ values (closed symbols) of CH_4 measured in cave air from different locations (see Fig. 3) are plotted as circles (Narrows, Pool and Boxing Ring sites), squares (Gib04a site), triangles (Lake site) and diamonds (Dark Rift site) with background atmosphere (labelled 'atm') plotted for reference. Part filled symbols are the air compositions interpreted as containing a biogenic methane input, see Fig. 5 and text for discussion. Cave environment data from (Matthey et al., 2010) augmented with new unpublished data.

mirrors a rise in CO_2 over the same period (Matthey et al., 2010).

The $\delta^{13}\text{C}$ of methane in soil pore space is plotted in Fig. 4c. Soil methane is mostly isotopically heavier than background atmospheric methane. This is consistent with isotopic fractionation resulting from loss of ^{12}C associated with methane consumption by methanotrophic bacteria (Whiticar, 1999). However, some measured soil methane samples have lighter $\delta^{13}\text{C}$ values than background atmosphere, suggesting sporadic production of biogenic methane forming under anaerobic conditions. This process that will be discussed in more detail below.

3.3. CH_4 in New St. Michaels Cave air

Reversing seasonal ventilation displaces CO_2 -rich cave air with CO_2 -poor background atmosphere during the summer months (Matthey et al., 2010), and can be clearly seen as cycles in CO_2

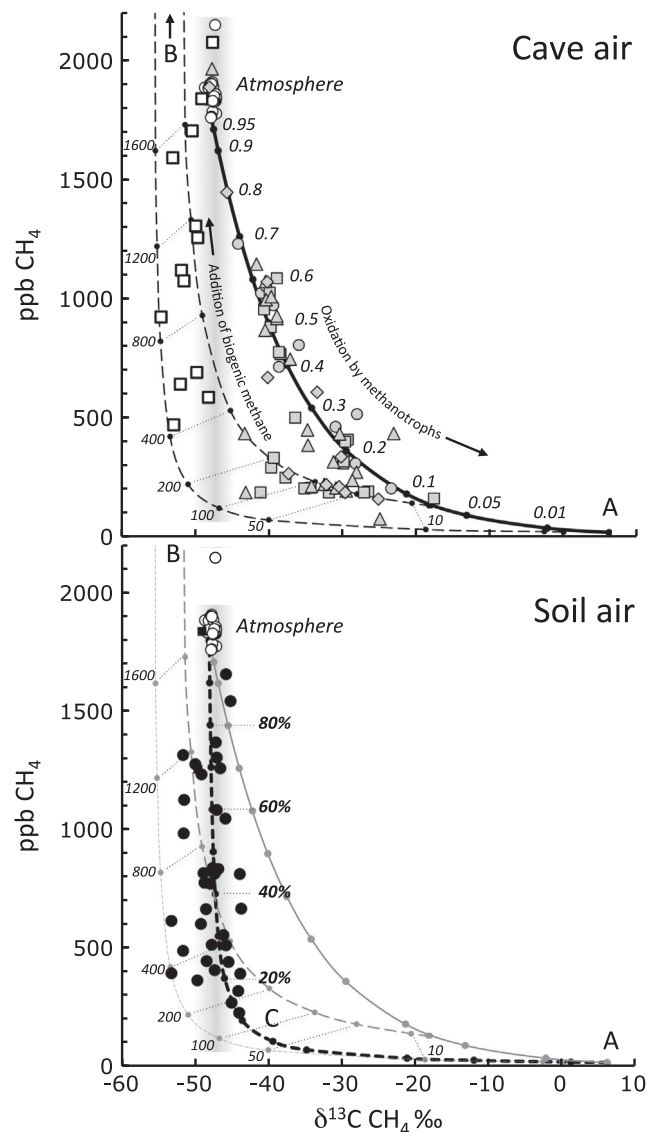


Fig. 6. Relationships between methane concentration and $\delta^{13}\text{C}$ in background atmosphere, cave and soil air using data from Figs. 4 and 5 collected monthly from 2004 until 2008 with addition data for air samples collected at less frequent intervals until 2010. Data for background atmosphere (open circles) and a shaded zone separating biogenic methane ($\delta^{13}\text{C} < -48\%$) from methane involved in bacterial oxidation ($\delta^{13}\text{C} > -48\%$) are shown for reference. The upper figure shows data for cave air as two groups: cave air with $\delta^{13}\text{C}$ values that are higher than background atmosphere which define an inverse correlation with abundance (circles: Narrows, Pool and Boxing Ring sites; squares: Gib04a site; triangles: Lake site) and cave air with 'biogenic' $\delta^{13}\text{C}$ values that are lower than background atmosphere (open squares). Curve A shows the locus of compositions formed by methanotrophic consumption of atmospheric methane modelled as a Rayleigh process using a kinetic fractionation factor of 1.012. Labels show the fraction (F) of atmospheric methane remaining in cave air. Addition of methane from a biogenic source can be modelled as mixing between atmosphere and a highly ^{12}C depleted residue of methanotrophic oxidation (curves B). Two mixing curves are shown which bound the data: the upper curve shows the effects of mixing between cave air with the most fractionated methane $\delta^{13}\text{C}$ value (130 ppb CH_4 , $\delta^{13}\text{C} = -18\%$) and a pure biogenic source with a $\delta^{13}\text{C} = -54\%$; the lower curve represents the effects of mixing between a highly fractionated residual methane formed at the end of curve A (20 ppb CH_4 , $\delta^{13}\text{C} = 0\%$) and a pure biogenic source with a $\delta^{13}\text{C} = -56\%$. Labels show the fraction in ppm of biogenic methane in cave air. Data for soil air are shown in the lower figure (closed circles). Curves A (methane consumption) and B (biogenic addition) are shown for comparison. Soil data define another mixing relationship, curve C, which is a simple two component mixing between atmosphere and air containing highly fractionated residual methane formed at the end of curve A (20 ppb CH_4 , $\delta^{13}\text{C} = 0\%$). Labels to the right of curve C show the percentage of atmospheric-sourced methane present in soil air samples. See text for discussion.

abundance that are synchronous with seasonal temperature in Fig. 5a and b. Methane abundances in cave air show an inverse relationship to the ventilation-controlled seasonal cycles shown by CO₂ (Fig. 5c). In summer, when CO₂ levels are lowest as a result of ventilation by CO₂-poor atmosphere, CH₄ levels are highest but usually still below background atmospheric levels. In winter, cave air methane concentrations fall to very low levels, typically less than 200 ppb (Fig. 5c). Thus the CO₂-rich ground air reservoir that in winter advects upwards and exhales out of entrances into the atmosphere is strongly depleted in CH₄.

The δ¹³C composition of cave air methane varies strongly with concentration (Fig. 5d). In winter CH₄ concentrations are generally low and δ¹³C values are higher than atmospheric air, reaching −15‰. This appears to reflect depletion of CH₄ from the outwards flowing cave air and by implication from the ground air reservoir that supplies this flow. In summer CH₄ concentrations are higher but generally below atmospheric levels and have higher δ¹³C values than atmosphere, generally −40 to −45‰. This pattern is consistent with CH₄ depletion of atmospheric air entering the cave. An exception occurs in summer 2007 when two samples of cave air have CH₄ characteristics that are indistinguishable from background atmosphere, indicating a period of exceptionally strong ventilation and less CH₄ depletion of the air as it flows into and through the cave.

There are sporadic occasions when measurements do not fit this seasonal pattern. They are characterised by δ¹³C values lighter than that of background air that fall as low as −55‰ (grey symbols, Fig. 5c and d). They tend mainly to occur in winter and always contain higher concentrations of methane compared to other data in the same phase of the seasonal cycle. This is evidence of an additional, possibly biogenic source of cave methane.

4. Discussion

4.1. Sources of methane in cave air

The atmosphere of some hypogene caves contains unusually high levels of methane (up to 3%, e.g. Movile Cave (Hutchens et al., 2004)) related to the action of chemoautotrophic bacteria (Forti et al., 2001) but there are no equivalent macro-ecosystems associated with the St. Michaels cave systems and the data obtained in this study reflect the natural background behaviour of methane in a dynamically ventilated cave environment. In the case of CO₂, where concentrations are lower in background atmosphere than in the cave, ventilation results in dilution of the high levels of CO₂ found in ground or vadose air. The situation is reversed in the case of methane, which is removed by methanotrophic bacteria in soil (Whiticar, 1999) a process that is clearly confirmed by the analysis of CH₄ in soil air made in this study (Fig. 5). Thus air introduced into the cave via the soil zone and ground air reservoir in voids in the bedrock with small apertures would be CO₂-rich but methane levels would be lower than background atmosphere. Ventilation by atmosphere through cave openings and large fissures (i.e. limited or no interaction with soil or bedrock surfaces) would be expected to elevate methane levels back to near atmospheric concentrations while diluting levels of CO₂. Similar effects produced by entry of large airflows via fissures were demonstrated by Atkinson et al. (1983) using radon as a natural tracer in Castleguard Cave, Canada.

Methane concentrations in soil and cave air possess similar ranges in values that are significantly lower than background atmosphere. However it seems unlikely that methane in cave air undergoes gas exchange directly with the soil zone because monthly cave and soil air methane mixing ratios are uncorrelated. Cave air methane displays strong seasonality that is synchronous

but opposite to the seasonal pattern in pCO₂ (Fig. 5) and ventilation via direct connections to the atmosphere such as cave entrances is the dominant exchange pathway controlling cave air methane.

The δ¹³C variations in soil and cave air methane provide considerable insight into the nature of gas exchange between the atmospheric, soil and ground air methane reservoirs. Biological and thermogenic processes impart large kinetic fractions on the carbon isotopic composition of natural methane and the δ¹³C of atmospheric methane (≈−47‰) is a product of inputs from an isotopically wide range of sources (e.g. Denman et al., 2007). The isotopic composition of carbon in biogenic methane formed by methanogens is enriched in ¹²C relative to background atmosphere and varies from −110‰ to −50‰ (e.g. Schoell, 1988; Whiticar, 1999). Thermogenic methane and, pertinent to this study, the residual methane that remains after aerobic or anaerobic oxidation becomes progressively depleted in ¹²C such that soil gas methane is characterised by high δ¹³C values up to −20‰ (King et al., 1989; Tyler et al., 1994; Whiticar, 1999). Vadose zone waters in Gibraltar contain high levels of sulphate derived from marine aerosol (unpublished data) and this may be an electron acceptor that potentially would enable anaerobic oxidation of methane to occur. The magnitude of kinetic fractionation factors associated with methane oxidation vary between 1.009 in anoxic aqueous environments (Alperin et al., 1988) and 1.025–1.049 during gas transport in soils above landfill, the latter showing inverse variation with temperature (Chanton and Liptay, 2000).

The variations of δ¹³C as a function of methane concentrations in cave air (upper figure) and soil air (lower figure) are illustrated in Fig. 6. Background atmosphere forms a compact group of analyses with around 1800 ppb methane and a δ¹³C of −47‰ and defines a key reference point in the interpretation of the data in Fig. 6 which for the purpose of this discussion is used to separate the cave air data into two groups. Cave air having δ¹³C values that are higher than background atmosphere mostly define an inverse relationship with methane abundance, with δ¹³C rising to > −20‰ in air with lowest CH₄ concentrations (grey symbols, Fig. 6). Some samples with concentrations below 300 ppb and slightly lower δ¹³C values lie to the left of this trend and are discussed below. The strong depletion of ¹²C as a function of CH₄ abundance is diagnostic of methane consumption by methanotrophic bacteria and the data can be modelled by a Rayleigh process using a fractionation factor of 1.012 (curve A). The cave air samples with lowest CH₄ abundances are consistent with 90% removal of an atmospheric component by bacterial oxidation (Fig. 6). As seen in Fig. 5, the cave air shows regular seasonal variations in CH₄ content, and on Curve A in Fig. 6 the methane in summer cave air plots towards the atmospheric end member, whereas for winter the cave air methane plots closer to the distal end of the curve.

The second group of analyses (plotted as open squares in Fig. 6) comprises cave air samples that have methane δ¹³C values that are significantly lower than background atmosphere. These samples also stand out as having elevated CH₄ abundances relative to the normal seasonal pattern (Fig. 5). The low values of δ¹³C indicate that they contain methane from an additional biogenic source. The range of compositions that result from mixing biogenic methane with the ¹²C-depleted residue of microbial oxidation is also shown in Fig. 6 (curves labelled B). Two mixing curves are shown which bound the data: the upper curve shows the effects of mixing between cave air that lies on Curve A with the most fractionated measured methane δ¹³C value (130 ppb CH₄, δ¹³C = −18‰) and a pure biogenic source with a δ¹³C = −54‰ and the lower curve represents the effects of mixing between a more extremely fractionated (and hypothetical) residual methane formed at the end of curve A (20 ppb CH₄, δ¹³C = 0‰) and a pure biogenic source

with a $\delta^{13}\text{C}$ -56% . The fit of these mixing curves around the spread of data points with biogenic $\delta^{13}\text{C}$ values (shown as open squares) is insensitive to the precise composition of the methane-depleted end member but requires that the biogenic source has a compositional range of $-55 \pm 1\%$. Mixing of between 400 and 1600 ppm of pure methane to cave air accounts for the lower $\delta^{13}\text{C}$ values seen in the 'open square' samples that have an unambiguously biogenic signature relative to the data for external atmospheric air. Furthermore, mixing of strongly CH_4 -depleted cave air with much smaller additions of biogenic methane can clearly account for the compositions of the samples already noted as lying to the left of Curve A with cave air CH_4 concentrations below 300 ppb. Thus, the large range of CH_4 concentrations and $\delta^{13}\text{C}$ compositions observed in cave air can be interpreted in terms of two dominant processes, the first being progressive depletion of CH_4 from atmospheric air by microbial oxidation (Curve A), and the second being addition of traces of biogenic methane to strongly CH_4 -depleted air (Curves B). Further evidence supporting this hypothesis will be discussed below.

Methane in soil air displays rather different behaviour and is compared in Fig. 6 (lower figure) with the major trends shown by cave air. Soil air possesses a wide range of CH_4 abundances coupled to a much smaller variation in $\delta^{13}\text{C}$. Soil is an environment where methane is rapidly oxidised and is a methane sink operating on a global scale. The large range in methane abundances with no associated isotopic fraction in Gibraltar soil suggests that the samples of soil air are the result of mixing between atmosphere and air that has become extremely depleted in methane. Curve C (Fig. 6) is the locus defining mixing between atmosphere and the same end member residue of microbial oxidation used to calculate the lower curve B in the cave air diagram. The general trend of the soil air data is consistent with such mixing and with proportions of between 20% and 80% of atmosphere in the mixture. However some of the soil air samples also contain methane with $\delta^{13}\text{C}$ values lower than background atmosphere which is indicative of a biogenic input. These compositions can be clearly identified in the time series plot in Fig. 4c and show a consistent relationship to the biogenic mixing model (curves B) in Fig. 6. They lie in the field between the two curves B, suggesting that they could represent additions of small amounts of biogenic methane to a highly CH_4 depleted soil gas, i.e. one which at the time contained very little unmodified atmospheric air. However it is uncertain that this is really the explanation, because the biogenically influenced soil air samples are not clearly separated as a group from the general scatter of soil air samples with $\delta^{13}\text{C}$ similar to atmosphere (marked out by the shaded areas in Fig. 6). An alternative explanation for their low $\delta^{13}\text{C}$ values is the addition of relatively large amounts of biogenic CH_4 to soil gases with compositions that lay on or close to the mixing line model shown as curve C in Fig. 6 (lower). Downwards transfer of atmospheric methane by diffusion is yet another process that would produce isotopic fractionation favouring lower $\delta^{13}\text{C}$ values, but to create the data array in Fig. 6 this fractionation would be need to be rather precisely balanced by the opposite isotope effect produced by soil methanotrophy.

Soil gases appear to be consistent with mixtures of two main components with probable sporadic additions of a third, namely biogenic methane. The two main components appear to be unaltered atmospheric air and air that has been extremely depleted in CH_4 by methanotrophs. The presence of such strongly contrasting gases in close proximity within the soil implies that both oxidation of methane and its biogenic formation may be taking place within micro-environments that are partially isolated from the majority of the pore space. Soils on the Upper Rock display moderate to well-developed crumb structure, and it is

tempting to speculate that the small pores within crumbs may provide such micro-environments while the larger pore spaces between crumbs contain largely unmodified atmospheric air, as do the cracks between peds. If so, CH_4 would diffuse from the large structural pores into the crumbs where its destruction by methanotrophs would act as a sink, resulting in very CH_4 -depleted gas with high $\delta^{13}\text{C}$ values within the intra-crum pore space. During sampling the gas pressure in both types of pores would be lowered by pumping, causing advective exchanges between the two, and the mixing of the CH_4 -depleted intra-crum gases with the dominant atmospheric air component in the larger pores, as they were drawn into the sampling device. Effectively, this picture is one of transport of CH_4 as a reactive trace gas through a double porosity medium, a situation that has well-studied analogues in hydrogeology and chemical engineering (Barker, 1985a, 1985b; Cushman, 1990; Dullien, 1991; Whitaker, 1988).

4.2. Biogenic sources of cave methane

The evidence for biogenic methane can be seen in the cave air data in Fig. 5 where a number of air collections contain methane that has $\delta^{13}\text{C}$ values lower than the $\delta^{13}\text{C}$ of the atmospheric background (-47.5%), and higher concentrations relative to the segment of the seasonal ventilation cycle. As noted above, these data, identified by open squares in Fig. 6, can be explained by mixing of trace amounts of ^{12}C enriched biogenic methane ($\delta^{13}\text{C} = -55 \pm 1\%$) with the ^{12}C -depleted residue of methanotrophic oxidation (curve B, Fig. 6). The cave air analyses with concentrations below 300 ppb which are displaced from the Curve A to more ^{12}C enriched compositions may also be explained by addition of very small amounts of biogenic methane (in this case less than 200 ppm). It is very interesting to note that many of these samples showing slight ^{12}C enrichment were collected on days when samples taken from other parts of the cave were either locally enriched in biogenic methane (i.e. paired with air samples that plot as open squares in Fig. 6) or on days when all the air in the deep cave had the same weak biogenic signature (i.e. paired with other samples in the group). Therefore the data provide compelling evidence of multiple sources of methane in cave air and that the systematic study of methane abundance vs. carbon isotope ratios provides a useful discriminant of methane sources and cave processes.

The mixing process defined by the isotopic data in the soil zone suggests a high degree of permeability that is unsurprising considering that the soil is both thin and becomes very dry during the summer drought. Anomalies in the soil and cave air data characterised by elevated CH_4 concentrations with $\delta^{13}\text{C}$ values lower than -47.5% again suggest the intermittent presence of a biogenic methane component. Soil permeability to gases and the gas-filled porosity might both become reduced during periods of heavy rain whereby exchange with atmosphere may become restricted and anoxic conditions favouring methanogenesis may temporarily develop. Overall the soil and cave air data suggest there are brief periods where anaerobic methanogenesis can occur both in the soil and elsewhere along pathways where methane of biogenic origin can enter the cave. Almost all of the cave air occurrences are in winter and spring, and there is weak evidence from the correspondence between these biogenic methane bursts and the rainfall records in Figs. 4 and 5 that suggests the anaerobic conditions develop during periods of high rainfall, perhaps as a result of high water content restricting gas circulation in the fractured bedrock as well as in the soil. However the disparity in time resolution between air sampled at monthly intervals and rainfall records taken daily makes this link tenuous at present.

4.3. Methane sources and sinks in the atmosphere–soil–karst system

The methane abundance– $\delta^{13}\text{C}$ relationships in cave air are controlled by microbial oxidation that seems to operate in a similar way during both summer and winter ventilation modes. The main difference is that CO_2 -rich ground air that is advecting out of the caves through upper entrances into the atmosphere during winter shows the largest depletion in methane relative to background atmosphere, with concomitant enrichment in $\delta^{13}\text{C}$ to values as high as -25‰ . Bacterial oxidation is likely to be taking place on biofilms on wet rock surfaces where methanotrophic bacterial reside. Moonmilk (Hill and Forti, 1997; Williams, 1959), a secondary calcite deposition associated with bacterial processes (Blyth and Frisia, 2008; Borsato et al., 2000; Northup et al., 2000) is present on some cave walls of New St. Michaels and these may be sites of methane oxidation. Lipids are commonly present in dripwater (e.g. Xiuli et al., 2011) and are indicative of microbes washed down from the soil zone. Accumulations of organic matter, including microbes, are likely to be present in the fractures and fissures in the bedrock itself. The extent to which bacterial oxidation in winter occurs in the ground air filled voids and fissures of the limestone bedrock, or on the cave walls of larger chambers is uncertain at present. However it is significant that summer ventilation, displacing cave air with CH_4 -rich background atmosphere, restores methane levels to around only 60% of the atmospheric background concentration. This could be a consequence of remixing of new atmosphere with old ground air but the isotopic compositions of summer cave air methane falls along the same oxidation curve A as does winter air in Fig. 6, and are not displaced to the atmospheric mixing curve B (Fig. 6) that defines the soil environment. This suggests that bacterial oxidation of new atmospheric methane may be taking place as air is drawn into the cave indicating that methane consumption may be a rapid process taking place on damp rock surfaces of the cave passages. A similar process was proposed to explain rapid fluctuations of methane mixing ratios in cave air monitored by FTIR spectroscopy over a three week period (Waring et al., 2009) where methane concentrations followed a diurnal pattern from normal atmospheric 1700 ppb to < 200 ppb which was also negatively correlated with CO_2 . Significantly methane concentrations were observed to cycle by up to 1000 ppb in only a few hours also suggesting that bacterial consumption is very rapid process (Waring et al., 2009). The cave air exhaled in winter shows the greatest degree of methane depletion and this is a consequence of a much longer residence time as ground air in the bedrock fissures and voids, where constant temperatures and humidity and greater rock surface areas per unit volume of gas favour more complete oxidation.

A conceptual model of methane dynamics in cavernous karst is illustrated in Fig. 7. The methane reservoirs of interest are represented by atmosphere, soil air, cave air and ground air. Ground air is the CO_2 -rich air reservoir permeating the unsaturated zone (Atkinson, 1977a). Because of density driven seasonal advection of air through the bedrock, it acts both as a source and a sink for the gas reservoir in cave air. Cave air is linked to the background atmosphere via two pathways. One of these passes through the soil zone and forms the ground air source for cave air, the other is a direct link representing ventilation through cave entrances. The main methane sources considered here are atmosphere (1800 ppb) and biogenic sources created under wet anoxic conditions. Elsewhere, additional methane sources e.g. from ecosystems may also be significant inputs. Evidence of transient inputs of ^{12}C -enriched biogenic methane in both soil and cave air revealed in this study suggests that biogenic inputs are from both the soil zone and the bedrock ground air reservoir. Both sources are the result of microbial decay of organic matter,

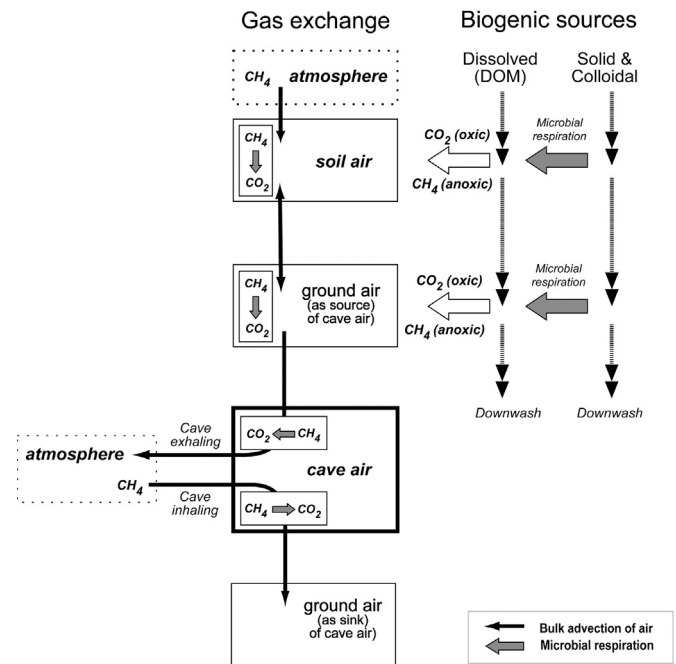


Fig. 7. A conceptual box model representing methane dynamics, sources and sinks in Gibraltar karst. See text for discussion.

the latter washed down into the unsaturated zone where we suggest that anoxic conditions may develop locally under water saturated conditions and temporarily enrich methane in the ground air source. Otherwise, under normal oxic conditions CO_2 is produced in both environments, each contributing to the ground air CO_2 reservoir. Thus ground air is depleted in methane relative to background atmosphere as a result of (1) exchange with methane depleted soil air and (2) oxidation of incoming atmospheric CH_4 in the cave reservoir when in summer advection mode. In winter advection mode, methane depleted ground air is exhaled either directly to the atmosphere through cave entrances, or by permeation through the soil zone, providing further opportunities for methane stripping. Further work is needed to establish the relative fluxes and kinetics of the processes in this model to establish the net methane flux resulting from exchange between atmosphere and the unsaturated zone, which, as discussed below, could have significance regarding the atmospheric methane budget.

4.4. Wider implications for the atmospheric methane budget

The results of this study show that dynamically ventilated cave systems similar to Gibraltar act locally as a net sink for atmospheric methane which is inhaled into the caves and bedrock of the unsaturated zone, stripped by bacterial oxidation and then exhaled back to the atmosphere as CO_2 -rich, CH_4 -poor cave air. Although at present the full magnitude of such a karst methane sink is unknown, it is possible that if repeated across cave systems globally it may impact on the atmospheric methane budget. This study has been carried out on a cave system where vertical relief and positions of cave entrances induce vigorous seasonal ventilation that draws down background atmosphere during the summer and exhales cave air back into the atmosphere during the winter season. Seasonal ventilation patterns are recorded from caves elsewhere (e.g. Atkinson et al., 1983; Fairchild and Baker, 2012) but may operate in a different sense. Many caves which do not develop sustained chimney ventilation between multiple entrances still undergo ventilation exchange with atmosphere during specific seasons (Fairchild and Baker, 2012). In caves where

a single cave entrance lies at or close to the lowest point of the system (e.g. Obir Cave in Austria, Spötl et al. (2005)), outside air often flows into the entrance in winter and out of it in summer, the reverse of the pattern at NSM. Wherever ventilation enhanced gas exchange between atmosphere and the unsaturated zone is taking place, bacterial action may also be stripping atmospheric methane out of cave air. Since limestone occupies 13% of the global ice-free land surface, wherever karstic permeability and topography are favourable, dynamically ventilated karst may be sequestering atmospheric methane with potential implications for the global methane budget.

At present it is impossible to quantify the magnitude of a karst methane sink without a detailed assessment of the global capacity for gas exchange between the unsaturated zone and the atmosphere. New St. Michael's Cave in Gibraltar represents a particularly clear case in which topography, position of caves and seasonal climate drive powerful and persistent circulation of atmospheric air through the rock. The strength of advection driven by seasonality diminishes in the tropics but the global extent of karstified limestone and the vast numbers of caves therein warrant further investigation as to their potential impact on the atmospheric methane budget.

5. Conclusions

St. Michael's Cave, Gibraltar has been the subject of a comprehensive cave monitoring programme since 2004 to improve understanding of the cave processes and how they are related to the surface environment. This study shows that methane is a useful tracer of cave ventilation pathways where $\delta^{13}\text{C}$ variations in soil and cave air methane provide considerable insight into the nature of gas exchange between the atmospheric, soil and ground air methane reservoirs. Methane concentrations measured in soil air are lower than background atmosphere (as they are elsewhere) and samples extracted from the shallow soil in this study represent a mixture of atmosphere and a highly ^{12}C depleted residue of methanotrophic oxidation. Methane abundances in cave air are uncorrelated with soil zone methane but show an inverse relationship to the ventilation-controlled seasonal cycles shown by CO_2 . Cave air CH_4 abundances are strongly inversely correlated with $\delta^{13}\text{C}$ which rise to $> -20\text{‰}$ in cave air with lowest CH_4 concentrations. The strong depletion of ^{12}C as a function of CH_4 abundance is diagnostic of methanotrophy and can be modelled as a Rayleigh process with a kinetic fractionation of 1.012. Anomalies in the seasonal soil and cave air data patterns, characterised by elevated CH_4 mixing ratios with $\delta^{13}\text{C}$ values lower than -47‰ , provide evidence of the intermittent presence of biogenic input. The isotopic signature of microbial oxidation is present in cave air samples taken when the cave is ventilating in both summer and winter modes. In winter, CO_2 -rich ground air that advects out of the caves into the atmosphere is strongly depleted in methane but summer ventilation, displacing cave air with CH_4 -rich background atmosphere, restores methane levels to around only 60% of the atmospheric background concentration. The isotopic compositions of this 'new' methane retain the signature of methanotrophy and suggests that microbial oxidation may be a rapid process. This study provides evidence that the dynamically ventilated Gibraltar caves act as a net sink for atmospheric methane which is inhaled into the caves and bedrock of the unsaturated zone, stripped of methane by bacterial oxidation and then exhaled back to the atmosphere as CO_2 -rich, CH_4 -poor cave air.

Acknowledgements

This work was supported NERC Grants NE/D005280/1 and NE/G007292/1. We are indebted to Leslie Linares, Gibraltar Ornithological and Natural History Society for carrying out a vegetation survey at the

cave site. We thank Euan Nisbet for valuable insights and two anonymous referees who helped improve the manuscript.

References

- Alperin, M.J., Reebergh, W.S., Whiticar, M.J., 1988. Carbon and hydrogen isotope fractionation resulting from anaerobic methane oxidation. *Global Biogeochem. Cycles* 2 (3), 279–288.
- Atkinson, T.C., 1977a. CO_2 in the atmosphere of the unsaturated zone: an important control of groundwater hardness in limestones. *J. Hydrol.* 35, 111–123.
- Atkinson, T.C., 1977b. Diffuse flow and conduit flow in limestone terrain in the Mendip Hills, Somerset (Great Britain). *J. Hydrol.* 35, 93–110.
- Atkinson, T.C., Smart, P.L., Wigley, T.M.L., 1983. Climate and natural radon levels in Castleguard Cave, Columbia Icefields, Alberta, Canada. *Arct. Alp. Res.* 15 (4), 487–502.
- Baldini, J.U.L., 2010. Cave atmosphere controls on stalagmite growth rate and paleoclimate records. In: Rogerson, M. (Ed.), *Tufas and Speleothems: Unravelling the Microbial and Physical Controls*, vol. 336. Geological Society of London Special Publications, pp. 283–294.
- Banner, J.L., Guilfoyle, A., James, E.W., Stern, L.A., Musgrove, M., 2007. Seasonal variations in modern speleothem calcite growth in Central Texas, U.S.A. *J. Sediment. Res.* 77, 615–622.
- Barker, J., Fritz, P., 1981. Carbon isotope fractionation during microbial methane oxidation. *Nature* 293, 289–291.
- Barker, J.A., 1985a. Block geometry functions characterising transport in densely fissured media. *J. Hydrol.* 77, 263–279.
- Barker, J.A., 1985b. Modelling the effects of matrix diffusion on transport in densely fissured media. In: *Hydrogeology in the Service of Man, Memoirs 18th Congress of International Association of Hydrogeologists*. Cambridge, pp. 250–269.
- Benavente, J., Vadillo, I., Carrasco, F., Soler, A., Liñán, C., Moral, F., 2010. Air carbon dioxide contents in the Vadose Zone of a Mediterranean Karst. *Vadose Zone J.* 9, 126–136.
- Blyth, A.J., Frisia, S., 2008. Molecular evidence for bacterial mediation of calcite formation in cold high-altitude caves. *Geomicrobiol. J.* 25, 101–111.
- Borsato, A., Frisia, S., Jones, B., van der Borg, K., 2000. Calcite moonmilk: crystal morphology and environment of formation in caves in the Italian Alps. *J. Sediment. Res.* 70, 1179–1190.
- Bourges, F., Mangin, A., d'Hulst, D., 2001. Le gaz carbonique dans la dynamique de l'atmosphère des cavités karstiques: l'exemple de l'Aven d'Orgnac (Ardèche). *Earth Planet. Sci.* 333, 692–696.
- Chanton, J., Liptay, K., 2000. Seasonal variation in methane oxidation in a landfill cover soil as determined by an in situ stable isotope technique. *Global Biogeochem. Cycles* 14, 51–60.
- Cicerone, R.J., Oremland, R.S., 1988. Biogeochemical aspects of atmospheric methane. *Global Biogeochem. Cycles* 2, 299–327.
- Cushman, J.H., 1990. *Dynamics of Fluids in Hierarchical Porous Media*. Academic Press, London 505 pp.
- Darling, W.G., Goody, D.C., 2006. The hydrogeochemistry of methane: evidence from English groundwaters. *Chem. Geol.* 229, 293–312.
- Denman, K.L. et al., 2007. Couplings Between Changes in the Climate System and Biogeochemistry. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, pp. 499–587.
- Dlugokencky, E.J., Bruhwiler, L., White, J.W.C., Emmons, L.K., Novelli, P.C., Montzka, S.A., Masarie, K.A., Lang, P.M., Crotwell, A.M., Miller, J.B., Gatti, L.V., 2009. Observational constraints on recent increases in the atmospheric CH_4 burden. *Geophys. Res. Lett.* 36, article number L18803.
- Dullien, F.A.L., 1991. *Porous Media: Fluid Transport and Pore Structure*, 2nd ed. Academic Press, London 574 pp.
- Ek, C., Gewalt, M., 1985. Carbon-dioxide in cave atmospheres—new results in Belgium and comparison with some other countries. *Earth Surf. Processes Landforms* 10, 173–187.
- Fairchild, I.J., Baker, A., 2012. *Speleothem Science. From Process to Past Environment*. Wiley-Blackwell 450 pp.
- Fisher, R., Lowry, D., Wilkin, O., Srisankarajah, S., Nisbet, E.G., 2006. High-precision, automated stable isotope analysis of atmospheric methane and carbon dioxide using continuous-flow isotope-ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* 20, 200–208.
- Ford, D.C., Williams, P.W., 2007. *Karst Hydrology and Geomorphology*. John Wiley and Sons; Chichester, England.
- Forti, P., Galdenzi, S., Sarbu, S.M., 2001. The hypogenic caves: a powerful tool for the study of seeps and their environmental effects. *Cont. Shelf Res.* 22, 2373–2386.
- Hill, C., Forti, P., 1997. *Cave Minerals of the World*. National Speleological Society, Huntsville, Alabama 446 pp.
- Hutchens, E., Radajewski, S., Dumont, M.G., McDonald, I.R., Murrell, J.C., 2004. Analysis of methanotrophic bacteria in Miville Cave by stable isotope probing. *Environ. Microbiol.* 6, 111–120.
- King, S.L., Quay, P.D., Lansdown, J.M., 1989. The $^{13}\text{C}/^{12}\text{C}$ kinetic isotope effect for soil oxidation of methane at ambient atmospheric concentrations. *J. Geophys. Res.* 94, 18273–18277.
- Linares, L., Harper, A., Cortes, J., 1996. *The Flowers of Gibraltar. Flora Calpensis. Gibraltar Botanic Gardens* 196 pp.

- Lowry, D., Holmes, C.W., Rata, N.D., O'Brien, P., Nisbet, E.G., 2001. London methane emissions: use of diurnal changes in concentration and $\delta^{13}\text{C}$ to identify urban sources and verify inventories. *J. Geophys. Res. D: Atmos.* 106, 7427–7448.
- Matthey, D., Lowry, D., Duffet, J., Fisher, R., Hodge, E., Frisia, S., 2008. A 53 year seasonally resolved oxygen and carbon isotope record from a modern Gibraltar speleothem: reconstructed drip water and relationship to local precipitation. *Earth Planet. Sci. Lett.* 269, 80–95.
- Matthey, D.P., Fairchild, I.J., Atkinson, T.C., Latin, J.-P., Ainsworth, M., Durell, R., 2010. Seasonal microclimate control of calcite fabrics, stable isotopes and trace elements in modern speleothem from St. Michaels Cave, Gibraltar. In: Rogerson, M. (Ed.), *Tufas and Speleothems: Unravelling the Microbial and Physical Controls*, vol. 336. Geological Society of London Special Publications, pp. 323–344.
- Miller, G.H., Mack, K.A., Dissly, R., White, J.W.C., Dlugokencky, E.J., Tans, P.P., 2002. Development of analytical methods and measurements of $^{13}\text{C}/^{12}\text{C}$ in atmospheric CH_4 from the NOAA Climate Monitoring and Diagnostics Laboratory Global Air Sampling Network. *J. Geophys. Res. D: Atmos.* 107 11–11–15.
- Northup, D.E., Dahm, C.N., Melim, L.A., Crossey, L.J., Lavoie, K.H., Mallory, L., Boston, P.J., Cunningham, K.I., Barn, S.M., 2000. Evidence for geomicrobiological interactions in Guadalupe caves. *J. Cave Karst Stud.* 62, 80–90.
- Raghoebarsing, A.A., Pol, A., Pas-Schoonen, K.T.v.d., Smolders, A.J.P., Ettwig, K.F., Rijpstra, W.I.C., Schoute, S., Damsté, J.S.S., Camp, H.J.M.O.d., Jetten, M.S.M., Strous, M., 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* 440, 918–921.
- Rodríguez-Vidal, J., Cáceres, L.M., Finlayson, J.C., Gracia, F.J., Martínez-Aguirre, A., 2004. Neotectonics and shoreline history of the Rock of Gibraltar, southern Iberia. *Quat. Sci. Rev.* 23, 2017–2029.
- Rose, E.P.F., Rosenbaum, M.S., 1991. *A Field Guide to the Geology of Gibraltar*. The Gibraltar Museum.
- Roslev, P., Iversen, N., Henriksen, K.H., 1997. Oxidation and assimilation of atmospheric methane by soil methane oxidizers. *Appl. Environ. Microbiol.* 63, 874–880.
- Sarbu, S.M., Kane, T.C., Kinkle, B.K., 1996. A chemoautotrophically based cave ecosystem. *Science* 272, 1953–1955.
- Schoell, M., 1988. Multiple origins of methane in the Earth. *Chem. Geol.* 71 (1–10), 71.
- Shaw, T.R., 1953a. New St. Michaels Cave. *BSA Cave Sci.* 3, 249–266.
- Shaw, T.R., 1953b. Old St. Michaels Cave. *BSA Cave Sci.* 3, 298–313.
- Spotl, C., Fairchild, I.J., Tooth, A.F., 2005. Cave air control on dripwater geochemistry, Obir Caves (Austria): Implications for speleothem deposition in dynamically ventilated caves. *Geochim. Cosmochim. Acta* 69, 2451–2468.
- Thauer, F.K., Moller-Zinkhan, D., Spormann, A.M., 1989. Biochemistry of acetate catabolism in anaerobic chemotrophic bacteria. *Annu. Rev. Microbiol.* 43, 43–50.
- Thorntonwaite, C.W., 1954. *The Measurement of Potential Evapotranspiration*. John P. Mather, Seabrook, New Jersey 225 pp.
- Treatman, E.K., 1971. The formation of the Gibraltar Caves. *Trans. Cave Res. Group G. B.* 13, 135–143.
- Tyler, S.C., Crill, P.M., Brailsford, G.W., 1994. $^{13}\text{C}/^{12}\text{C}$ fractionation of methane during oxidation in a temperate forested soil. *Geochim. Cosmochim. Acta* 56, 1625–1633.
- Waring, C., Griffith, D.W.T., Wilson, S., Hurrey, S., 2009. *Cave Atmosphere: A Guide to Calcification and a Methane Sink*. Goldschmidt Conference Abstracts, A1419.
- Wheeler, D., 2006. The Gibraltar climatic record: Part 1—the history of weather observations. *Weather* 61, 36–39.
- Whitaker, S., 1988. Diffusion in packed beds of porous particles. *Am. Inst. Chem. Eng. J.* 34, 679–683.
- White, W.B., 1969. Conceptual models for karst aquifers. *Ground Water* 7, 15–21.
- Whiticar, M.J., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chem. Geol.* 161, 291–314.
- Williams, A., 1959. The formation and deposition of moonmilk. *Trans. Cave Res. Group G. B.* 5 (2), 133–138.
- Wood, B.D., Petraitis, M.J., 1984. Origin and distribution of carbon dioxide in the unsaturated zone of the southern High Plains of Texas. *Water Resour. Res.* 20, 1193–1208.
- Xiuli, L., Canfa, W., Junhua, H., Chaoyong, H., Shucheng, X., 2011. Seasonal variation of fatty acids from drip water in Heshang Cave, central China. *Appl. Geochem.* 26, 341–347.