

Spatial variability of cave-air carbon dioxide and methane concentrations and isotopic compositions in a semi-arid karst environment

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Abstract There is insufficient information on the movement of air in karst environments to constrain the uncertainty associated with quantifying sources and sinks of methane (CH₄) and carbon dioxide (CO₂) within caves for global carbon accounting. We analysed cave-air samples for their CO₂ and CH₄ concentrations ([CO₂] and [CH₄]) and carbon isotopic compositions from sampling campaigns in winter (August 2014) and summer (February 2015) at numerous heights and locations throughout Gaden and Cathedral caves, in a semi-arid environment, Wellington Caves, NSW, Australia. Ventilation is the dominant control on cave-air CO₂ and CH₄, with the highest cave-air CO₂ concentrations ([CO₂]_{cave}) occurring in summer, in association with the lowest cave-air CH₄ concentrations ([CH₄]_{cave}). Analyses show that the cave-air CO₂ has both atmospheric and soil sources. Soil air and cave air in both caves undergo methanogenesis and methanotrophy, but we identify cave-specific differences in cave-air CH₄ and CO₂. [CH₄]_{cave} in Cathedral Cave shows an inverse relationship to [CO₂]_{cave}, particularly in areas separated from the main cave passage. In contrast, Gaden

Cave has near-atmospheric [CH₄]_{cave} and isotopic ratios present at all locations sampled in winter. Where no ventilation is occurring in summer, [CH₄]_{cave} in Gaden Cave decreases, but remains reasonably high compared to Cathedral Cave. Our research shows adjacent caves vary in their ability to act as a net sink for CH₄, and highlights the need for further studies before global generalisations can be made about the carbon budget of karst environments.

Keywords Karst · Methane sink · Semi-arid · Carbon dioxide · Caves · Isotopic ratio

Introduction

Caves provide a unique underground environment for studying environmental processes. These processes include speleothem formation (Fairchild and Baker 2012), cave-air carbon dioxide (CO₂) production and movement (Baldini et al. 2008; Cuezva et al. 2011; Cowan et al. 2013; Garcia-Anton et al. 2014; Meyer et al. 2014), seasonal density-driven cave ventilation (Bourges et al. 2006; Kowalczyk and Froelich 2010; Breecker et al. 2012; Cowan et al. 2013; James et al. 2015), and spatio-temporal variability and consumption of cave-air methane (CH₄) (Waring et al. 2009; Matthey et al. 2013; Fernandez-Cortes et al. 2015). Understanding CH₄ and CO₂ in the terrestrial ecosystem has become increasingly important due to the steady rise in atmospheric greenhouse gas concentrations (Houghton et al. 1996; Kirschke et al. 2013). The atmospheric concentration of CH₄ ([CH₄]_{atm}) has more than doubled since pre-industrial times (Kaplan et al. 2006). Studies in Mediterranean (Matthey et al. 2013) and oceanic climates (Garcia-Anton et al. 2014) indicate that CO₂ concentrations in caves [CO₂]_{cave} are inversely correlated with [CH₄]_{cave}

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(Fernandez-Cortes et al. 2015). Such a relationship results from both seasonal ventilation of cave-air and CH₄ production and oxidation in soils to produce CO₂ by methanotrophic bacteria. It has also been suggested that [CH₄]_{cave} is inversely proportional to cave-air ion concentration (Fernandez-Cortes et al. 2015). Fernandez-Cortes et al. (2015) hypothesise that radioactive radon (²²²Rn) decay could provide a source of hydroxyl radicals for the oxidation of cave-air CH₄, particularly where [CH₄] is too low to sustain methanotrophic communities. Cave-air isotopic analyses of CO₂ and CH₄ have been conducted in various caves (Fernandez-Cortes et al. 2015), however, further research is required from other latitudes. This will allow a statistical and spatially representative number of caves to determine the role of underground cave environments as CH₄ sinks on a global scale. Such studies are significant in determining the extent to which karst in general is to be considered a CH₄ sink.

Caves play a significant role in the reconstruction of palaeo-climates and palaeo-vegetation through the study of speleothems as an environmental proxy (Fairchild and McMillan 2007; Wainer et al. 2010; Fairchild and Baker 2012). Speleothems form from thin layers of calcite that have precipitated out of dripwater that has passed through soil and bedrock. Low cave-air CO₂ concentration compared to soil-air *p*CO₂ can influence speleothem formation by controlling the rate of dripwater degassing, causing calcite precipitation and speleothem growth (James et al. 2015). Studies have also demonstrated that [CO₂]_{cave} can affect seasonal variations in dripwater Mg²⁺/Ca²⁺ and Sr²⁺/Ca²⁺ ratios more than rainfall and drip rates (Wong et al. 2011). Knowledge of [CO₂]_{cave} at the Wellington Caves will contribute to the knowledge of speleothem formation and interpretations of past climates, particularly in semi-arid climate regions.

The aim of this research is to examine the spatial and vertical variability of the concentration and isotopic composition of CH₄ and CO₂ throughout Cathedral and Gaden caves located in Wellington Caves, NSW, Australia. These measurements are used to infer sources and sinks of CH₄ and CO₂, and mechanisms to explain changes. We analyse both summer and winter concentrations and δ¹³C values of CH₄ and CO₂ in background air, cave air and soil air, and δ¹³C–CO₂ values of limestone samples from the area to identify sources and processes of fractionation occurring within these gases in the karst environment.

Previous studies have shown that [CO₂] increases with distance into caves (Cowan et al. 2013; Fernandez-Cortes et al. 2015), and [CH₄] decreases correspondingly as CH₄ is oxidised and CO₂ is released in the cave or overlying soil and bedrock (Mattey et al. 2013, Fernandez-Cortes et al. 2015). Sources of CO₂ at Wellington Caves are likely to include organically derived CO₂ from soils, as has been

identified in similar studies (Baldini et al. 2006; Breecker et al. 2012; Meyer et al. 2014). There may also be a contribution from the local groundwater, which floods passages at the lowest parts of Cathedral Cave.

In addition to atmospheric CH₄, another minor source of cave-air CH₄ is organically derived CH₄ with δ¹³C values of approximately –60 ‰ (Whiticar 1999) when unaltered, or heavier depending on the extent of oxidation. Previous studies have indicated that water stores are present in the unsaturated zone above Cathedral Cave (Cuthbert et al. 2014a; Rutledge et al. 2014), which may provide an anoxic environment for methanogenesis to take place. These δ¹³C–CH₄ values, however, can be altered to a more ¹³C-enriched (higher) δ¹³C–CH₄ value if there is consumption of ¹²C by soil methanotrophs.

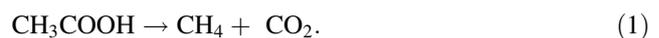
Isotopic fractionation and source signatures of CO₂

The fractionation of CH₄ and CO₂ C-isotopes during genesis, transport and consumption is significant for interpreting the processes that CO₂ and CH₄ have undergone before sampling and analysis (Fisher et al. 2011). These are discussed in detail in “Sources of CO₂ in caves”. It is possible to identify the source of CO₂ with the aid of Keeling (mixing) plots that graph δ¹³C versus the reciprocal of the total concentration of CO₂ (Keeling 1958, 1961; Pataki et al. 2003). A linear trend on a mixing plot indicates mixing between two end-members. As the cave is directly connected to the outside environment, one end-member is atmospheric air, which mixes with fractionated soil-air CO₂. Multiple samples from zones of anomalous CO₂ readings can be plotted to determine the source δ¹³C value, its origin, and any secondary fractionation processes that may have occurred. The ratios of C-isotopes are reported relative to the international standard of Vienna Pee Dee Belemnite (VPDB) in per mil notation (‰).

Sources of CO₂ in caves

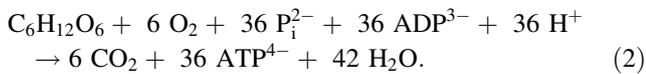
Soil

CO₂ forms in soils through the breakdown of organic matter in anoxic environments by bacteria, oxidation of CH₄ by methanotrophic bacteria, and via contribution from plant root and microbial respiration. The breakdown of organic matter by acetate fermentation produces CH₄ and CO₂ via the following pathway (Whiticar 1999):



Bacterial CO₂ produced in soils by the oxidation of glucose and fatty acids in organic matter produces CO₂ without a CH₄ by-product. This occurs in an aerobic

environment through the following glycolic pathway (Lodish et al. 2000):



The isotopic ratio ($^{13}\text{C}/^{12}\text{C}$) of the CO_2 produced is associated with the isotopic value of the C present in the organic matter of the soil, as well as any fractionation that occurs during organic matter processing by microbes (Baker et al. 2011). The C ratio in plants is controlled by the photosynthetic pathway [C_3 , C_4 or Crassulacean Acid Metabolism (CAM)] that the overlying vegetation types use (O'Leary 1981). C_3 plants incorporate atmospheric CO_2 by carboxylation of ribulose biphosphate, whilst C_4 plants incorporate CO_2 by carboxylation of phosphoenolpyruvate. CAM plants fix atmospheric CO_2 in a similar way to either the C_3 or C_4 method (O'Leary 1981), thus the isotopic ratios of CAM plants can vary widely. C_3 plants have a $\delta^{13}\text{C}$ value of approximately -25‰ , whilst C_4 plants have values of approximately -12‰ (Gleixner 2007). CAM plants have a $\delta^{13}\text{C}$ value of approximately -13‰ when C fixation occurs primarily at night, and -27‰ when there is no C fixation at night (O'Leary 1981). CO_2 associated with the breakdown of organic matter from a combination of these plants will therefore have a $\delta^{13}\text{C}$ value that is dependent upon the ratio of organic matter contribution from each plant type.

Soil properties are also likely to affect $[\text{CO}_2]$ and $[\text{CH}_4]$ in soil air caused by organic matter decomposition. For example, low nutrient levels, soil moisture and temperature can all affect CO_2 production in soils by limiting microbial activity (Fierer et al. 2003). Acidic soils have also been shown to reduce soil microbial activity and organic matter decomposition (van Bergen et al. 1998).

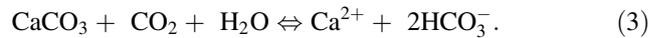
Ground-air CO_2

Evidence for ground-air CO_2 in the karst vadose zone below the soil was first reported by Atkinson (1977) and more recently by Ek and Godissart (2014). Ground-air CO_2 may be from continued microbial decomposition of organic matter leached from the soil. This organic matter is transported by infiltration of water through fractures in the limestone. Within the vadose zone, the organic matter decomposes by microbial digestion, producing air that is further enriched in CO_2 . Ground-air can then enter the cave via cracks and fissures in the bedrock, causing elevated $[\text{CO}_2]_{\text{cave}}$. The isotopic composition of ground-air CO_2 is, by its nature, difficult to measure directly. Unless the vadose zone contains organic matter that has a different isotopic composition from the modern-day soil, ground-air

CO_2 was expected to have a similar isotopic composition to that of the soil air.

Limestone

As water passes through limestone, it dissolves calcium carbonate (CaCO_3) via the following reaction (Suarez and Jurinak 2011):



This water makes its way into the cave due to gravitational forces. Upon entering the cave, if concentrations of dissolved CaCO_3 in this water are high enough, and cave-air $p\text{CO}_2$ is low enough, calcite precipitates and CO_2 degasses into the cave atmosphere. This degassed CO_2 is composed of a mixture of soil-air CO_2 , ground-air CO_2 and CO_2 from the degassing of dissolved limestone. Degassed CO_2 therefore has an isotopic composition intermediate to the biological and inorganic carbon end-members, and it typically has a lighter isotopic value than the water it has escaped from due to a preferential degassing of $^{12}\text{CO}_2$ during disequilibrium deposition (Dulinski and Rozanski 1990). Deininger et al. (2012) model the carbon isotopic composition of dripwaters and associated speleothems and infer an effect of $<1\text{‰}$ on speleothem $\delta^{13}\text{C}$ due to the effects of evaporation and ventilation over a wide range of drip rates (0–2000 s per drip). Degassed CO_2 may contribute to an increase in $[\text{CO}_2]_{\text{cave}}$. A light C isotopic value compared to the biological source in CO_2 air samples may suggest this origin.

External atmosphere

Atmospheric CO_2 enters cave systems predominantly during winter when the cave-air temperature is warmer than the external-air temperature and therefore less dense. Depending on cave morphology and the daily or seasonal ranges of external-air temperature (James et al. 2015), density-driven ventilation can cause cool atmospheric air to displace this warmer cave air. CO_2 accumulation is most likely to occur in underground caves that are vertical or sub-vertical and have a single entrance. The inverse of this trend is seen in caves with entrances at a lower elevation than the cave itself (James et al. 2015), for example those in a cliff face. Caves with large entrances that are highly exposed to the outside environment, allowing wind gusts to displace cave air, may also experience an inverse trend. The opposite of the winter ventilation trend is seen in New St. Michael's Cave in Gibraltar, where CO_2 -rich ground-air advects upwards and is discharged into the cave in winter. In summer, external air enters the cave and is distributed into the ground-air reservoir (Mattey et al. 2008, 2013).

Ventilation patterns in different environments vary as the stability of the external atmosphere varies. This is also important in determining the frequency, duration and intensity of cave ventilation. Environments with relatively stable daily temperatures are likely to experience predominantly seasonal cycles of ventilation. In environments with large variations in both daily and seasonal temperatures, 24-h cycles of ventilation may also be evident (James et al. 2015).

Groundwater

Groundwater is a significant source of CO₂ in other karst studies (Troester and White 1984). This occurs via the dissolution of limestone through the chemical pathway shown in Eq. 3. If the cave-air *p*CO₂ is low, CO₂-saturated groundwater is able to degas once it is exposed to the cave air.

The deepest accessible part of Cathedral Cave intercepts shallow groundwater at a steep drop in an area known as The Well. At times during the study, precipitated calcite sheets were seen floating on the water surface, indicating that the degassing of CO₂ from groundwater is a probable source in this area.

Magmatic sources

Mantle-derived CO₂ is also a possible source. Within the wider study region, a few effervescent bore water samples do show evidence of CO₂ from the mantle (Schofield and Janowski 2003, 2004). However, the groundwater at Cathedral Cave is a local connection to the adjacent alluvial river and groundwater system (Keshavarzi et al. 2014), and therefore CO₂ from the mantle is unlikely to be a source.

Study site description, cave morphology and local climate

Site description and geology

The study sites are Cathedral and Gaden tourist caves at Wellington Caves (Fig. 1a, b), New South Wales, Australia (32°37'14.88"S, 148°56'17.16"E). The site is an area of ongoing intense geological research (Osborne 2001, 2007), vertebrate palaeontology research (Čerňanský and Hutchinson 2013), dripwater and speleothem research (Jex et al. 2012; Cuthbert et al. 2014a, b; Rutledge et al. 2014; Rau et al. 2015) and biomarker research (Blyth et al. 2014). The caves were formed in a north–south trending ridge of Middle-Devonian Garra Formation limestone. This limestone formation is the remnant of approximately 1 km of material deposited in a shallow reef, which formed around a chain of volcanic islands off eastern Australia (Strusz

1965). The region was subsequently folded in the early Carboniferous (Strusz 1965). The main chamber of Cathedral Cave was formed along a boundary of massive lime-mudstone and folded thinly bedded limestone, which dips to the east (Osborne 2001). The local groundwater is intercepted at the end of the cave.

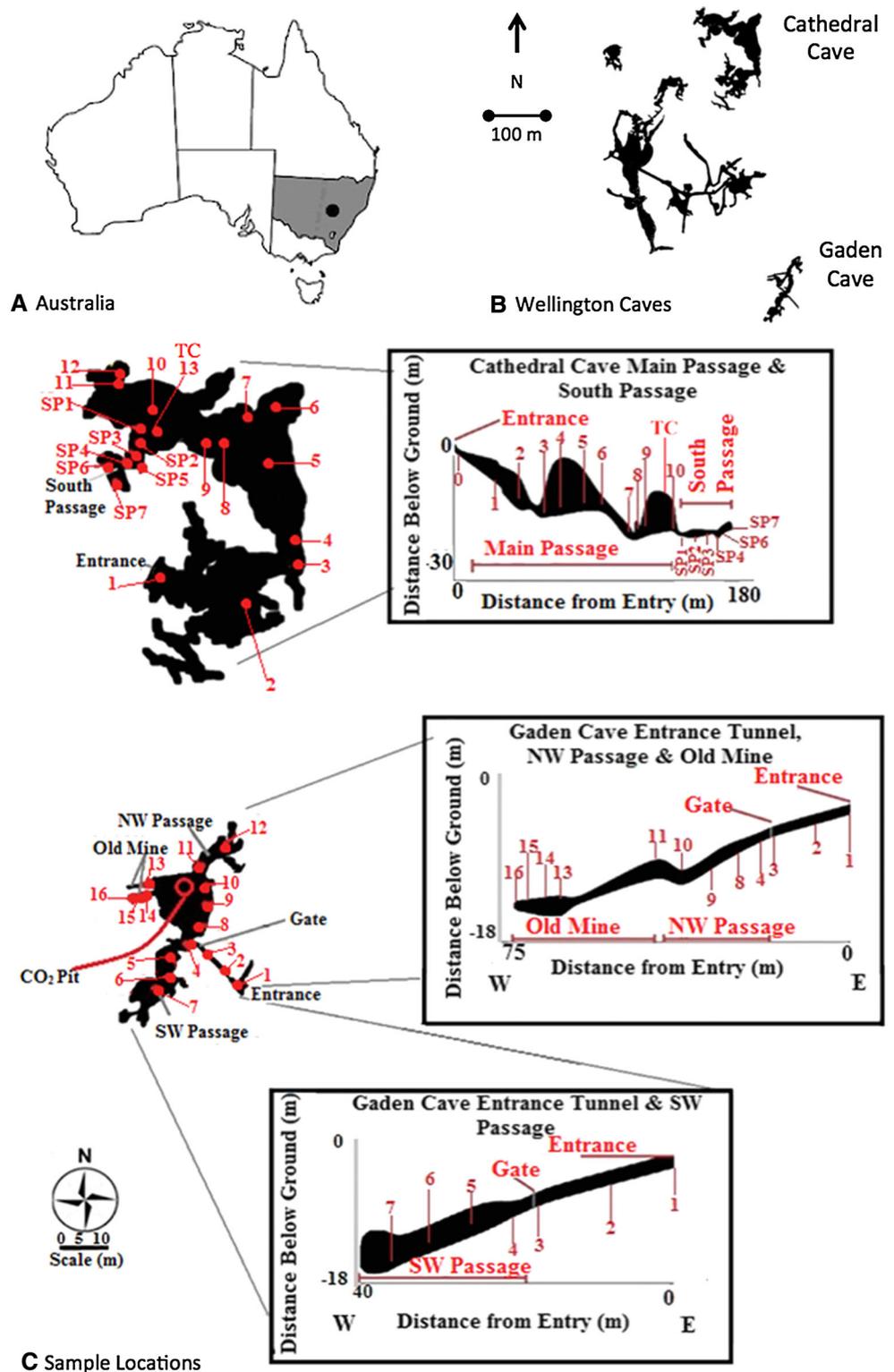
Cave morphology and climate

Figure 1c shows the morphological differences between Gaden Cave and Cathedral Cave. Gaden Cave features two major passages that split from the main entrance at the end of an excavated entrance tunnel. Both passages trend downwards; the south passageway ends in a chamber approximately 35 m from the cave entrance. The north passageway continues into a section known as “Old Mine”, which diverts from the end chamber and leads to the “CO₂ pit”. Excluding the “CO₂ pit”, Gaden Cave has a depth of approximately 25 m from cave floor to overlying soil surface. The maximum roof height is approximately 5 m in the end chamber of the north passageway. The passages are narrow (roughly 4 m wide and 5 m high) in contrast to Cathedral Cave. Gaden Cave is typically dry, with water only infiltrating in extremely wet years (the last time in 2010/2011).

Cathedral Cave has passages in the main chamber that reach 15 m wide and over 10 m high. It has been classified as a reactivated hypogene cave (Osborne 2007). A number of small tunnels divert to the sides from the main passage. The large passageway ends with exposed groundwater at The Well, approximately 150 m from the entrance and 30 m below the soil surface. Immediately south of The Well is a passageway known as South Passage. Much of the actively depositing stalagmites and stalactites of Cathedral Cave are found here (Osborne 2007; Jex et al. 2012). This indicates an active water supply to this part of the cave: dripwaters are active throughout the year and have been observed to be dripping continuously over time.

The climate of Cathedral Cave has been monitored at several locations during 2014 (Rau et al. 2015). Furthest from the cave entrance, in South Passage, air temperature is stable throughout the year at 17.8 °C with only minor fluctuations of ~0.1 °C, but close to the entrance temperature fluctuations of 2–5 °C are observed. Near-entrance cave-air temperatures are stable in summer, when cave-air temperatures are cooler than the external-air temperature. Daily variations in maximum cave-air temperatures in winter are evident near the entrance of Cathedral cave and are indicative of ventilation occurring at a daily timescale when external-air temperatures are lower than inside the cave (Figure S.11). Cave-air relative humidity was also relatively constant throughout the year at depth (97.1 %, with a range of 96.5–97.1 %). Close to the entrance, relative humidity varied substantially

Fig. 1 **a** Location of Wellington Caves, NSW. **b** Relative location of Cathedral and Gaden caves. **c** Diagrams of Cathedral and Gaden caves showing sampling locations and cross sections (modified from mapping and cave surveys performed by I. Cooper, P. Maynard, K. Moore, G. Ryan and K. Vaughan-Taylor). *Scale* refers to dimensions of caves only and does not represent the distance between the caves



(minimum, maximum and median values of 59.3, 97.9 and 88.6 %, respectively), again indicative of the replacement of high humidity cave air in winter due to ventilation. Temperature data from the continuous monitoring of cave-air temperature in Cathedral Cave was obtained from Rau

et al. (2015). These data indicate that during 2013 and 2014 ventilation in Cathedral Cave began in late March, reaching a maximum air exchange between 07/08/2014 and 12/08/2014. The cave returned to stable conditions in November during both years.

Above-ground vegetation and soil

An above-ground survey of plant species was performed over the entire area of Gaden (approximately 40 × 60 m) and Cathedral caves (approximately 80 × 90 m) to account for any potential effects of vegetation types on soil-air CO₂ by plant respiration and organic matter breakdown. The largest and most predominant vegetation type over the extent of both Cathedral and Gaden caves is C₃ eucalyptus. Both caves are located below a number of large, though sparse, C₃ *Callitiris* pines. Soil above Cathedral Cave is likely to have some organic matter contribution from the CAM plant *Agave Americana*, which populates the areas beside and above the entrance to Cathedral Cave. Many *Bidens pilosa* (C₃) plants are located above the north and north-eastern sections of Gaden Cave as well as the eastern and western sections of Cathedral Cave. The soil type located above both caves is a red loam with a shallow A horizon above a B horizon (Frank 1971) with a total thickness ranging from a few centimetres to approximately 1 m. This soil is composed predominantly of clays (60 %), as well as quartz, chert and calcite nodules with a median grain size of <2 μm (Frank 1971).

Local climate

The average annual rainfall since recording began at Agrowplow, Wellington in 1882, is 615.9 mm (weather station 65,034, <http://www.bom.gov.au>). Temperature at Wellington varies widely from between 2.9 and 16.0 °C in winter and 16.2 and 32.1 °C in summer (based on average minimum and maximum temperatures at weather station 65,034, <http://www.bom.gov.au>). Inter-annual rainfall variability is high, with mean annual rainfall typically below average since 2000, with the exception of a wet year in 2010 where total yearly rainfall exceeded 1000 mm. The maximum and total rainfall during winter sampling (15/08/14–22/08/14) were 14.4 and 22.2 mm, respectively. The maximum and total rainfall during summer sampling (6/02/15–13/02/15) occurred on the 1 day and was 5.6 mm.

Soil temperature and moisture levels during sampling were obtained from the on-site weather station (data available from <http://groundwater.anu.edu.au/monitoring-station/42>). The average soil temperature in August 2014 was 9.1 °C with a maximum, minimum and standard deviation of 17.2, 3.6 and 3.0 °C, respectively. During February 2015 the average soil temperature was 26.7 °C with a maximum, minimum and standard deviation of 40.6, 15.4 and 5.3 °C, respectively. Volumetric soil moisture levels at 30–60 cm depth during August 2014 and February 2015 averaged 37 and 21 %, respectively, with standard deviations of 0.01 in both months. Soil moisture values at 30–60 cm depth were more stable during both periods at

the site than surface soil moisture levels (10 cm depth), which displayed comparable average values for each month but with larger ranges and standard deviations.

Materials and methods

Cave-air, soil-air, external-air and limestone sampling

Cave-air sampling

Air samples were taken at 1 m intervals vertically, from the ground up to 4 m in height, both above the cave entrance (for external atmosphere air samples), and throughout the cave in winter 2014 (August) and summer 2015 (February). Air samples were collected using both a low flow SKC pump and a 2 L sterile syringe (SGE Analytical Science). Air was pumped into and stored in 3 L Tedlar bags, which were stored in a cool location with no exposure to sunlight. Samples were analysed the following week. The syringe and pump were attached to non-reactive Teflon tubing (5 mm internal diameter), which was taped to a pole at 1 m intervals such that the samples collected at 4 m height passed through 4 m of Teflon tubing before entering the syringe or pump.

The total numbers of cave visitors in Gaden Cave were 269 and 68 during August 2014 and February 2015, respectively. The total numbers of visitors to Cathedral Cave were higher: 861 during August 2014 and 480 during February 2015. This equates to daily averages of 9 and 28 visitors to Gaden and Cathedral caves, respectively, in August 2014, and 2 and 17 visitors to Gaden Cave and Cathedral Cave, respectively, in February 2015. As tourist numbers at these caves are very low, it is unlikely that cave tours and human respiration would cause any significant increase in [CO₂]_{cave} during sampling. Other studies such as Faimon et al. (2006) have also reported that elevated [CO₂] from cave tourists dissipates rapidly. Furthermore, a study performed by Cowan et al. (2013) found no increases in [CO₂] caused by the operator taking samples, and that [CO₂]_{cave} fluctuations were seasonal and not correlated with cave visitation rates. Nonetheless, to minimise the risk of contamination, where possible sampling was done either before or well after daily cave tours took place. As a further precaution, one person was sent ahead to sample.

Soil-air sampling and 24-h CO₂ concentration measurements

Gradient soil-air point samples, collected by inserting Teflon tubing into the soil, were chosen over surface chamber measurements. This was to eliminate the chance that the chamber may create a microenvironment, changing

the pressure and temperature compared to the outside climate and giving $\delta^{13}\text{C}$ readings that do not accurately represent the environment. Studies indicate that standard deviations for source signatures are lower for point samples where the air is pumped directly into Tedlar bags as opposed to chamber measurements (Srikantharajah et al. 2012). Soil-air samples for Cathedral Cave in summer and winter and Gaden Cave in summer only were collected by the methods outlined for pre-2007 soil-air sampling in Matthey et al. (2013). Teflon tubing was inserted into the soil profile at three depths (0–10, 10–20, 20–30 cm) using a hollow steel rod. The soil was allowed to resettle around the tubing before sampling. The sampling tube was flushed for 2 min before pumping the soil air into Tedlar bags. Soil samples were taken from the same soil profiles after soil air was sampled and stored in ziplock bags for analysis.

Limestone sampling

Massive and thinly bedded limestone samples were taken from two sites above Wellington Caves (32°37'32.9"S, 148°56'19.6"E and 32°37'23.76"S, 148°56'14.52"E). To obtain these samples, outcrops of massive and thinly bedded limestone were broken with a hammer to expose an unweathered surface. Powder was ground from the unweathered surface of the limestone using a micro-drill and transferred to a 2 mL Eppendorf Safe-Lock tube.

Data analysis and quality control

Limestone sample IRMS analyses

Limestone samples were analysed for their C isotopic compositions by Isotope Ratio Mass Spectrometry (IRMS) with a Kiel carbonate device at the University of New South Wales' (UNSW) Mark Wainwright Analytical Centre. IRMS analyses were in dual-inlet mode against CO_2 reference gas (Thermo Fisher Scientific) and using NBS-18 and NBS-19 as primary standards. The reference gas contains isotopic ratios of -4.5‰ ($\delta^{13}\text{C}$) and -14.1‰ ($\delta^{18}\text{O}$) at 70 °C. The precision for $\delta^{13}\text{C}-\text{CO}_2$ for each sample is 0.1 ‰.

Analysis of soil microbiology

DNA was extracted from 5 g of soil from each soil layer using phenol–chloroform extraction as described by Lueders et al. (2004). Subsequently, the DNA was precipitated using polyethylene glycol 6000 (Sigma Aldrich), and the DNA pellet was washed once with 70 % (v/v) ethanol and resuspended in 50 μL nuclease free water (Qiagen). DNA concentration and purity were determined by standard agarose gel electrophoresis and

fluorometrically using RiboGreen (Qubit Assay Kit, Invitrogen) according to the manufacturer's instructions. The extracted DNA was used as target for Illumina sequencing. Amplicon libraries were generated by following Illumina's 16S Metagenomic Sequencing Library Preparation Protocol, using 12.5 ng of template DNA per reaction. PCR cycles for the initial PCR were reduced to 21 to avoid PCR biases from over-amplification. The following universal primer pair was used for the initial amplification, consisting of an Illumina-specific overhang sequence and a locus specific sequence:

```
926F_Illum(5'-TCGTCGGCAGCGTCAGATGTGT
ATAAGAGACAG[AAACTYAAAKGAATTGRCC
G]-3'),
1392R_Illum(5'-GTCTCGTGGGCTCGGAGATGT
GTATAAGAGACAG[ACGGGCGGTGTGTRC]-3').
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This universal primer pair targets the V6–V8 hyper-variable regions of the 16S Ribosomal RNA gene and has been shown to capture the microbial diversity of Bacteria and Archaea in a single reaction (Wilkins et al. 2013). PCR products were purified using a magnetic bead capture kit from Agencourt AMPure XP beads, Beckman Coulter and quantified using a fluorometric kit (RiboGreen, Qubit Assay Kit, Invitrogen). Purified amplicons were subjected to the Index PCR using the MiSeq platform (Ramaciotti Centre for Genomics, UNSW Australia) according to the manufacturer's instructions. Illumina sequences were checked for quality (FastQC, BaseSpace) and analysed using the BaseSpace cloud computing platform (<http://basespace.illumina.com>) and MOTHUR (<http://www.mothur.org/>) with modified protocols (Schloss et al. 2009; Kozich et al. 2013). Taxonomy was assigned against the GreenGenes Database (<http://greengenes.secondgenome.com>). To ensure even sampling depth for subsequent analyses, OTU abundance data was rarified to the lowest number of sequences for a sample (7301 sequences per sample).

Cave- and soil-air sample analyses

Air samples were analysed using Picarro G1101-*i* (CO_2) and Picarro G2132-*i* (CH_4) Cavity Ring Down Spectrometers (CRDS) at UNSW. Standards of Southern Ocean Air (supplied by the Commonwealth Scientific and Industrial Research Organisation (CSIRO)) were frequently measured to quantify drift throughout the CO_2 and CH_4 air sample analyses. Due to limited availability, standard 1 ($\delta^{13}\text{C}-\text{CO}_2 = -10.5\text{‰}$, $[\text{CO}_2] = 415.24\text{ ppm}$, $\delta^{13}\text{C}-\text{CH}_4 = -47\text{‰}$, $[\text{CH}_4] = 1.87\text{ ppm}$) was run sporadically during analyses. Standard 2 ($\delta^{13}\text{C}-\text{CO}_2 = -10.5\text{‰}$, $[\text{CO}_2] = 393.43\text{ ppm}$, $\delta^{13}\text{C}-\text{CH}_4 = -47\text{‰}$, $[\text{CH}_4] = 1.77\text{ ppm}$) was run between every 5–6 samples

for both CO₂ and CH₄ analyses. Standards 1 and 2 were analysed for their [CH₄] by CSIRO using a gas chromatograph (GC) with flame ionisation detection (FID) and reported on the National Oceanic and Atmospheric Administration's (NOAA) NOAA04 CH₄ scale (Dlugokencky et al. 2005). [CO₂] was measured by CSIRO using FID after methanisation of CO₂ to CH₄ and reported in the WMOX2007 CO₂ mol fraction scale (Dlugokencky et al. 2005). Relativity to the scale was established with 10 primary standards (ranging from 290 to 420 ppm CO₂) calibrated by the NOAA. International calibration of the CSIRO air is monitored by continuous comparisons with standards (WMO Round Robins, IAEA CLASSIC, and cylinder exchanges with NOAA), as well as monthly comparisons with samples collected at Cape Grim. δ¹³C–CO₂ values of the CSIRO standards are reported against the international VPDB–CO₂ scale via an internal scale, with samples measured using a dual-inlet Finnigan MAT252 stable isotope ratio mass spectrometer.

All Picarro G2132-*i* δ¹³C–CH₄ values for this study were corrected against an offset obtained by measuring samples on both the Picarro G2132-*i* and a Gas Chromatography Isotope Ratio Mass Spectrometry (GC-IRMS) system at Royal Holloway, University of London, UK (RHUL). δ¹³C–CH₄ values for standard 1 were compared against the value obtained for standard 1 by RHUL. The median offset of UNSW values was –1.68 ‰ during August sample analyses and –0.40 ‰ for February sample analyses. These values were <1.2 ‰ different from the median offset of 94 samples dual-analysed by GC-IRMS (RHUL) and by the Picarro G2132-*i* at UNSW from March 2014 to September 2014 (Iverach et al. 2015). Therefore, drift was minimal and the measurements were not fluctuating beyond the specifications of 24 h drift for the machine. Thus the correction based on the 94 samples analysed by RHUL used by Iverach et al. (2015) was used to correct for the error seen in higher δ¹³C–CH₄ values. The precision of the Picarro G2132-*i* is 0.8 ‰ for 5-min analyses with a 2.0 ‰ 24-h drift. The maximum within-day and between-day variations for standards 1 and 2 observed for the Picarro G2132-*i* during cave-air analyses were both <2.0 ‰.

CO₂ air samples were analysed with the Picarro G1101-*i* with standard 1 (August samples only) and standard 2 run between every 5–6 samples. All sample and standard bags were shaken prior to and during analysis to ensure CO₂ did not settle in the Tedlar bag. Samples anticipated to have [CO₂] higher than the range of linearity of the instrument (~3500 ppm) were diluted with zero-CO₂ air before analysis. As standard 1 was not consistently available for February analyses, δ¹³C–CO₂ values were corrected by obtaining the median daily δ¹³C–CO₂ values for standard 2

and correcting against the offset between the known (CSIRO) and daily median δ¹³C–CO₂ value. For the August samples, the shift of the median values obtained at UNSW for standards 1 and 2 was averaged and this was used as the correction factor for δ¹³C–CO₂ values. Maximum within- and between-day variance of all analysed δ¹³C–CO₂ values was <0.5 ‰. Precision over 5 min for analyses on the Picarro G1101-*i* is <0.3 ‰ (1σ). The maximum range of values for both standards seen within a day (or group of days where correction was done in multiple-day groups) for the Picarro G1101-*i* was 1.2 ‰ with a standard deviation of 0.48 ‰ (observed for standard 2 values analysed on the 24/02/15).

The 2 L sampling syringes (SGE Analytical Science) used to collect air samples were tested for any potential fractionation effects of δ¹³C for both CO₂ and CH₄. Standard Southern Ocean air (CSIRO) was used to fill unused Tedlar bags (*n* = 5). The bags were then analysed for their CO₂ and CH₄ isotopic compositions on both the Picarro G1101-*i* (CO₂) and the Picarro G2132-*i* (CH₄). Each bag was shaken before air was extracted into the syringe and then expelled out into the atmosphere to flush the syringe. The remaining air in the bag was syringed out and reinserted into the same bag. The bags were then re-analysed for isotopic compositions of CO₂ and CH₄. The difference between the δ¹³C–CO₂ averages for the syringed samples and the original bag samples was –0.7 ‰. The difference between the δ¹³C–CH₄ averages was –0.4 ‰. For both machines the standard deviations of δ¹³C for the CH₄ and CO₂ values were lower between syringed samples than between those with the original standard. This confirmed that the syringe method was adequate for sampling the cave-air CO₂ and CH₄ without fractionation.

Mixing plots were used to determine the source of the CO₂ in each cave. The isotopic composition of each ambient air sample is assumed to be a combination of a background end-member (δ¹³C_(b); [CO₂ (b)]) and a point source end-member (δ¹³C_(s); [CO₂ (s)]). This isotopic composition is determined from (Keeling 1958, 1961; Pataki et al. 2003):

$$\delta^{13}\text{C}_{(a)} = \frac{[\text{CO}_{2(b)}] (\delta^{13}\text{C}_{(b)} - \delta^{13}\text{C}_{(s)})}{[\text{CO}_{2(a)}]} + \delta^{13}\text{C}_{(s)}, \quad (4)$$

where δ¹³C_(a) and [CO_{2(a)}] are the isotopic value and concentration measured in the ambient air sample, respectively. The isotopic signatures in sections of each cave were determined by fitting Eq. 4 to the data using a non-linear model fitting function in Mathematica (Wolfram Research, Inc. 2012).

A Rayleigh (1896) distillation model was fitted to the cave-air CH₄ data to establish the rate of oxidation of CH₄. The system is approximated by:

$$\delta^{13}\text{C}-\text{CH}_{4\text{loc}} \approx 1000 \left\{ \left[\frac{1}{\alpha} - 1 \right] \times \ln\left(\frac{c_{\text{loc}}}{c_{\text{start}}}\right) \right\} + \delta^{13}\text{C}-\text{CH}_{4\text{start}} \quad (5)$$

where c_{start} is the $[\text{CH}_4]$ at location 0, c_{loc} is the $[\text{CH}_4]$ at a location in the cave, $\delta^{13}\text{C}-\text{CH}_{4\text{start}}$ is the isotopic composition at location 0, and $\delta^{13}\text{C}-\text{CH}_{4\text{loc}}$ is the isotopic composition at a location in the cave (Coleman et al. 1981; Grant and Whiticar 2002). The fractionation factor (α) for the oxidising CH_4 was determined by fitting Eq. 5 to the data using a non-linear equation fitting function in Mathematica (Wolfram Research, Inc. 2012). Equation 5 was fixed to pass through the median atmospheric value for air in rural NSW (Iverach et al. 2015; Kelly et al. 2015), whilst allowing α to float. Reported α values for microbial oxidation range from 1.002 to 1.035 (Grant and Whiticar 2002; Matthey et al. 2013).

To explore patterns in the data for both caves, a principal component analysis (PCA) was performed on the CO_2 and CH_4 concentration and isotopic composition data. Samples with missing data were excluded and the PCA performed on the remaining 164 data points in Microcal Origin. The data were normalised before the PCA.

Results

Limestone

IRMS results identified a $\delta^{13}\text{C}$ value of $2.9 \pm 0.1 \text{ ‰}$ for thinly bedded limestone and $1.5 \pm 0.1 \text{ ‰}$ for massive limestone at Wellington Caves.

Soil CO_2 and CH_4

The concentration of CO_2 measured from soil-air Tedlar bag samples from above Cathedral Cave ranged between 4737 to 8544 ppm (August) and 710 to 1203 ppm (February). Mixing plots of winter (Figure S.1) and summer (Figure S.2) soil-air samples indicate biological soil CO_2 source signatures of -22.5 ‰ (95 % CI: -23.2 to -21.9 ‰) in August and -18.7 ‰ (95 % CI: -19.8 to -17.7 ‰) in February. Concentrations of CH_4 in soil-air above Cathedral Cave ranged from 0.89 to 1.03 ppm (August) and 1.45 to 1.65 ppm (February). These concentrations suggest that this soil is a sink for biologically derived CH_4 . Methanotrophic activity and associated CO_2 production in this soil are more dominant in winter due to higher rainfall, which results in increased microbial and root respiration. In summer, there is less methanotrophic activity, resulting in higher $[\text{CH}_4]$ and correspondingly lower $[\text{CO}_2]$ than in winter. This is due to reduced microbial and root respiration as a result of decreased

moisture content and high temperatures (average $31.4 \text{ }^\circ\text{C}$ over 3-day sampling period [weather station 65,034, <http://www.bom.gov.au>]). Due to pump failure after collection at Cathedral Cave, no soil-air samples were collected above Gaden Cave in winter. We were able to collect summer soil-air samples above Gaden Cave and the least squares best-fit of the mixing model had a biological CO_2 source signature of -19.5 ‰ (95 % CI: -19.8 to -19.1 ‰) (Figure S3).

Soil microbiology

Methanotrophic bacteria belonging to the Alphaproteobacteria, Gammaproteobacteria and Verrucomicrobia were observed in the microbial communities in the upper and lower soil layers (15.9 and 12.4 % relative abundance) above Cathedral Cave (Fig. 2). However, the species compositions of the methanotrophic communities were significantly different. The upper soil layer was dominated by methanotrophs within the Alphaproteobacteria (73 % of total methanotrophs) closely related to *Methylosinus* species (*Methylosinus acidophilus*, *Methylosinus pucelana*, *Methylosinus* sp.), *Methylocella* species (*Methylocella palustris*, *Methylocella silvestris*, *Methylocella* sp.) and *Methylobacterium* sp. whereas the lower soil layer harboured predominantly methanotrophs within the Gammaproteobacteria (*Methylophaga* sp., *Crenothrix* sp., and *Methylomicrobium* sp.). Both soil layers comprised sequences affiliated to *Methylacidiphilum* sp. within the phylum Verrucomicrobia.

Additionally, methylotrophic bacteria closely related to *Methyloversatilis*, *Methylobacillus*, *Methylotenera* (Betaproteobacteria) and *Hyphomicrobium* (Alphaproteobacteria) were observed, feeding partially on by-products of CH_4 oxidation, particularly methanol or formaldehyde. Previous studies have shown that these methylotrophs are observed in many different methanotrophic habitats accompanying methanotrophic bacteria (Hutchens et al. 2004; Saidi-Mehrabad et al. 2013; Kumaresan et al. 2014).

CH_4 -producing archaea (methanogens) were almost exclusively observed in the lower soil layer and were closely related to *Methanosaeta pelagica* and *Methanohalophilus mahii* within the order *Methanosarcinales* (Methanomicrobia) (Fig. 2). The relative high abundance of the obligate acetoclastic *Methanosaeta* sp. (5.1 % relative abundance) suggests the significance of acetate as an important metabolic intermediate in the lower soil horizon.

Throughout the caves there are aeolian deposits and conglomerates, as well as soil that has been brought into smooth tourist pathways. The microbiological communities within the cave would therefore be similar to those described in the overlying soil profile.

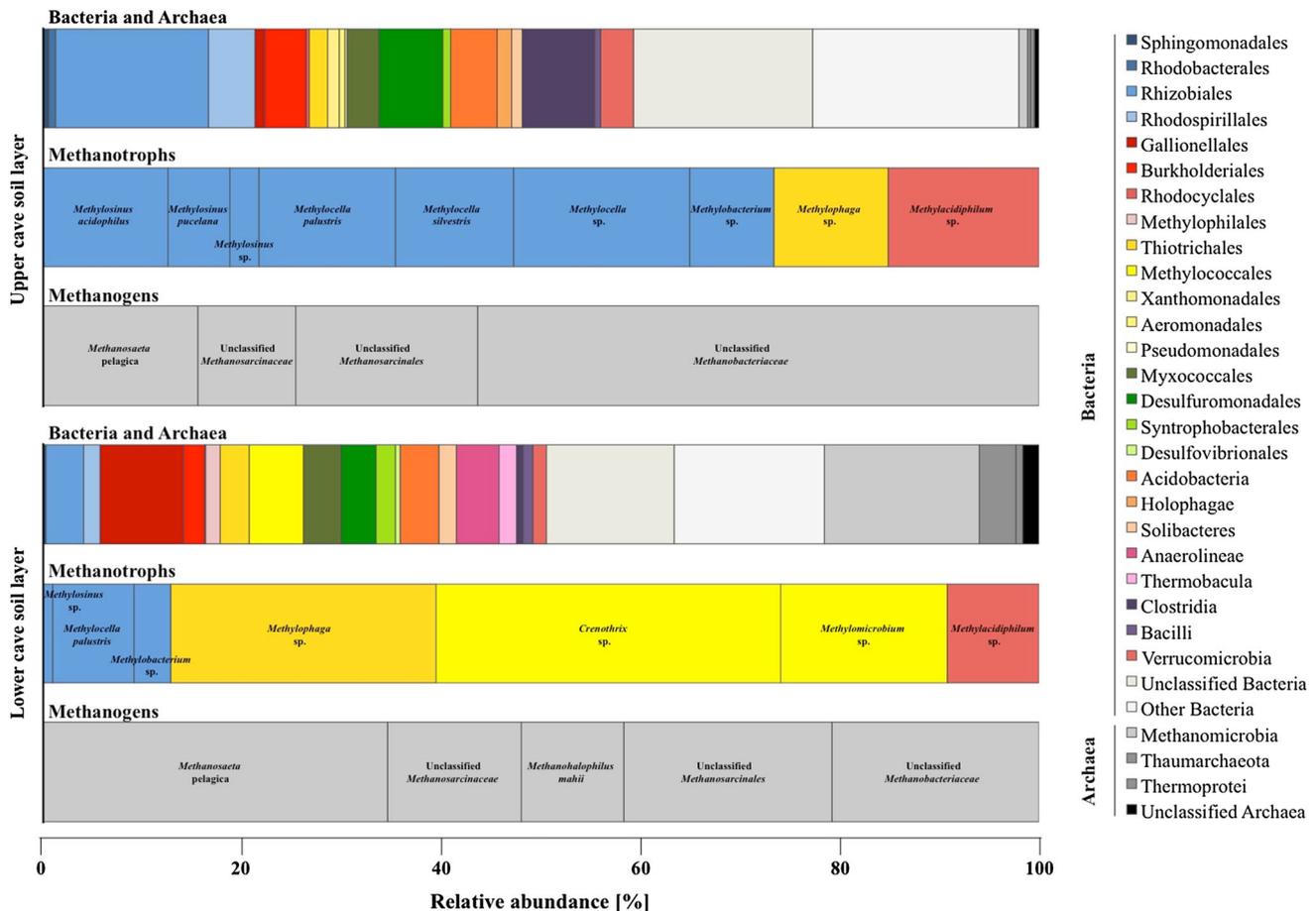


Fig. 2 Relative abundance of most common bacterial and archaeal taxa (GreenGenes Database; >0.2 %) in different soil layers in the Wellington Caves. The methanotrophic and methanogenic

community composition is highlighted by zooming out of the original microbial composition using *additional bars*

Cave-air and external-air CO₂ and CH₄

Cave-air and external-air CO₂ and CH₄ concentrations

The average [CO₂]_{atm} and [CH₄]_{atm} are 393.27 ppm ($n = 4$) and 1.75 ppm ($n = 4$), respectively. The average, median and standard deviation of [CO₂] and [CH₄] at Cathedral Cave and Gaden Cave in August 2014 and February 2015 are shown in Table 1.

During both summer and winter, the relationship between [CO₂] and distance from the entrance appears to be strongest in sections of the caves that have limited connection to the main passage(s), such as South Passage and Thunder Cave in Cathedral Cave, and Old Mine in Gaden Cave (see Figs. 3, 4c, 5c). Figures 4d and 5d show a slight increase in [CO₂] with increasing height from floor in both caves. Irrespective of season, [CO₂] and distance from entrance are more strongly correlated in Gaden Cave (winter $R^2 = 0.60$; $n = 31$; $p = 0.00$; summer $R^2 = 0.40$;

$n = 29$; $p = 0.00$) than Cathedral Cave (winter $R^2 = 0.12$; $n = 40$; $p = 0.03$; summer $R^2 = 0.21$; $n = 40$; $p = 0.00$).

The median [CH₄] of 1.79 ppm (winter) and 1.43 ppm (summer) in Gaden Cave is close to the background atmospheric concentration of 1.77 ppm (winter) and 1.73 ppm (summer) at the cave entrance. Near-atmospheric [CH₄] was measured at all locations within Gaden Cave in winter, irrespective of the distance from the cave entrance or height within the cave (see Fig. 5). In summer, near-atmospheric [CH₄] was measured at all locations in Gaden Cave except “Old Mine”. Irrespective of season, Cathedral Cave [CH₄] and distance from entrance are more negatively correlated (winter $R^2 = -0.39$; $n = 40$; $p = 0.00$, summer $R^2 = -0.54$; $n = 40$; $p = 0.00$), than in Gaden Cave (winter $R^2 = -0.17$; $n = 30$; $p = 0.03$, summer $R^2 = -0.27$; $n = 29$; $p = 0.00$).

Figure 4 shows that [CH₄] is inversely correlated with [CO₂] in Cathedral Cave (winter $R^2 = -0.45$; $n = 40$; $p = 0.00$; summer $R^2 = -0.43$; $n = 40$; $p = 0.00$),

Table 1 Average, median and standard deviation of [CO₂] and [CH₄] concentration and δ¹³C (‰) values at Cathedral Cave and Gaden Cave in winter/ August 2014 and summer/ February 2015

	Cathedral Cave		Gaden Cave	
	Conc. (ppm)	δ ¹³ C (‰)	Conc. (ppm)	δ ¹³ C (‰)
Average				
CO ₂	1530 (2975)	-14.57 (-15.84)	870 (5146)	-14.30 (-18.38)
CH ₄	1.48 (0.89)	-42.04 (-39.38)	1.78 (1.43)	-46.92 (-44.56)
Median				
CO ₂	665 (1004)	-13.63 (-15.73)	776 (2405)	-46.95 (-45.85)
CH ₄	1.69 (0.21)	-45.01 (-42.03)	1.78 (1.53)	-46.92 (-44.49)
Standard deviation				
CO ₂	2865 (4653)	2.93 (2.41)	486 (7359)	2.16 (2.55)
CH ₄	0.44 (0.50)	5.25 (7.61)	0.02 (0.21)	0.23 (3.27)

Summer 2015 values are shown in brackets

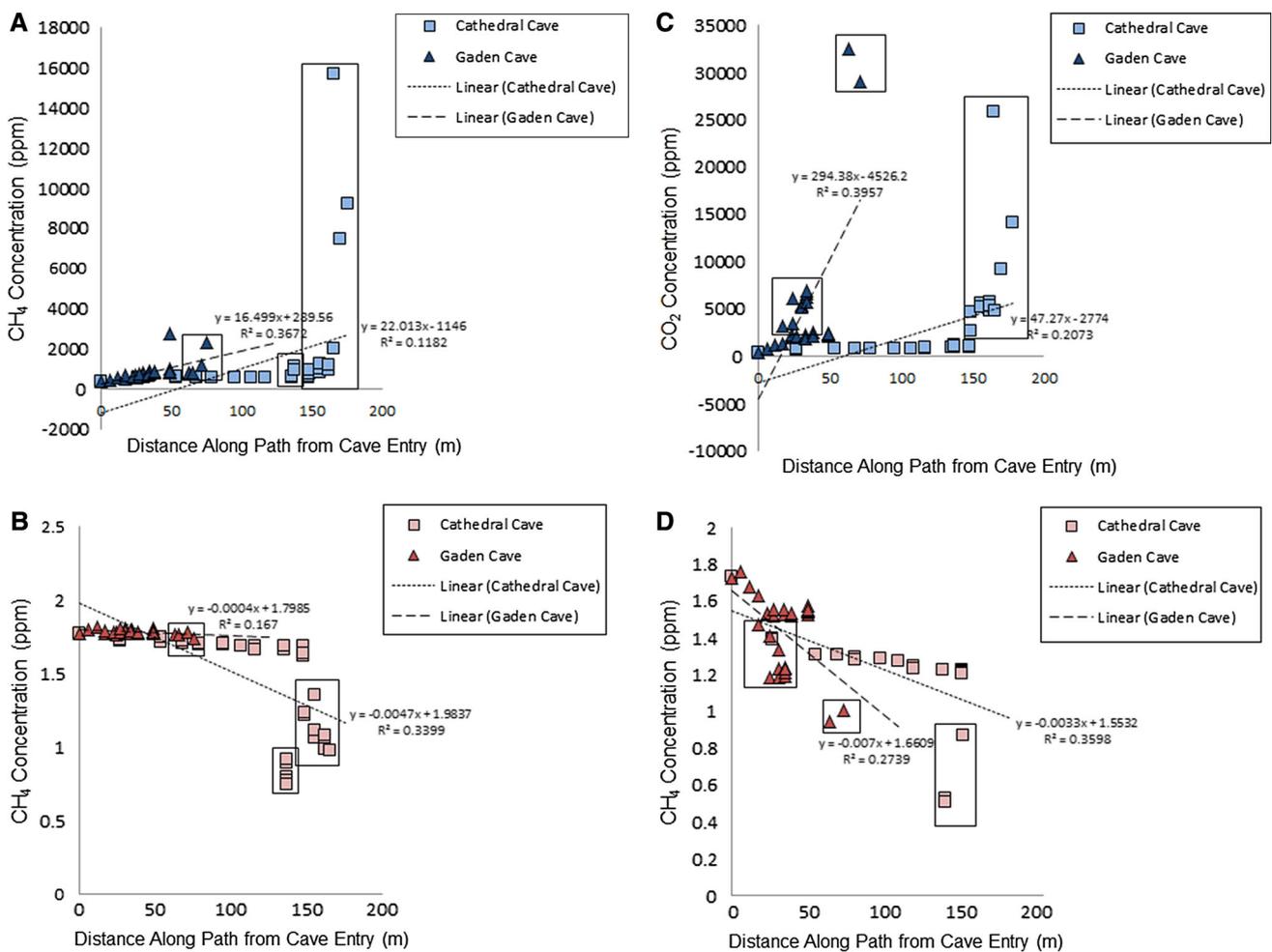


Fig. 3 CH₄ (red) and CO₂ (blue) concentrations in Gaden and Cathedral caves in August, 2014 (left) and February, 2015 (right) plotted against path distance from cave entrance

particularly during winter in South Passage, which branches off from the main passage (winter $R^2 = -0.66$; $n = 17$; $p = 0.00$). These findings agree with those reported in the literature (Mattey et al. 2013; Garcia-Anton

et al. 2014) although they are lower than reported in Fernandez-Cortes et al. (2015). There is also a significant inverse correlation in Gaden Cave in summer ($R^2 = -0.61$; $n = 29$; $p = 0.00$), however, there is no

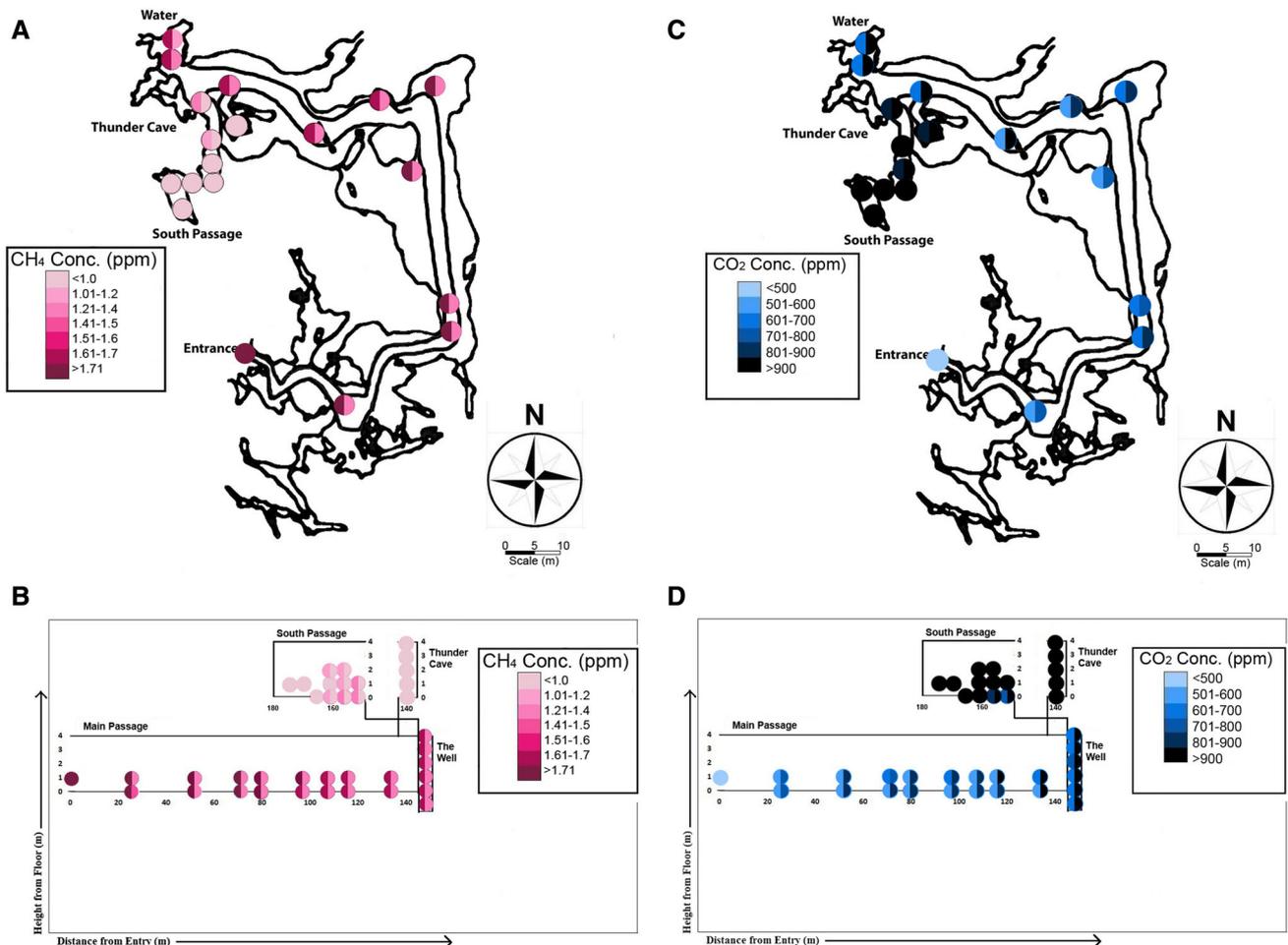


Fig. 4 Birds eye view diagrams showing CO_2 (a) and CH_4 (c) in Cathedral Cave at 1 m height from cave floor [values at water surface shown for the well (site 12)] and schematic diagrams showing CO_2

(b) and CH_4 (d) concentrations in Cathedral Cave. The left side of the coloured points indicates winter 2014 values. The right side indicates summer 2015 values

significant correlation in winter ($R^2 = -0.17$; $n = 30$; $p = 0.02$).

Cave-air and external-air CO_2 and CH_4 isotopic compositions

The average $\delta^{13}\text{C}-\text{CO}_2$ and $\delta^{13}\text{C}-\text{CH}_4$ values for external atmospheric air at Wellington Caves are -8.7‰ ($n = 4$) and -47.0‰ ($n = 4$), respectively. The CO_2 source signatures in Cathedral Cave and Gaden Cave main passages in winter were -21.6‰ (95 % CI -21.8 to -21.4‰) (Cathedral Cave, Figure S.4) and -20.9‰ (95 % CI -21.2 to -20.7‰) (Gaden Cave, Figure S.5), respectively. In summer, the CO_2 source signatures in Cathedral Cave and Gaden Cave were -21.5‰ (95 % CI -21.6 to -21.3‰) (Cathedral Cave, Figure S.6) and -21.1‰ (95 % CI -21.3 to -20.8‰) (Gaden Cave, Figure S.7). The average, median and standard deviation of CO_2 and CH_4 $\delta^{13}\text{C}$ values at Cathedral Cave and Gaden Cave in

August 2014 and February 2015 are shown in Table 1 and the values for the side passages are shown in Table 2.

CH_4 oxidation

Winter $\delta^{13}\text{C}-\text{CH}_4$ values in Gaden Cave are approximately external atmospheric levels, which indicates that the atmosphere is the only significant source of CH_4 (see Table 1). To explore CH_4 processes within the caves, both winter and summer CH_4 data were plotted in $\delta^{13}\text{C}-\text{CH}_4$ versus $[\text{CH}_4]$ graphs (Figures S.8, S.9 and S.10). The distribution of points within these graphs shows that, relative to ambient air outside of the caves, CH_4 is being oxidised within the caves, bedrock or overlying soils. We fitted a Rayleigh distillation model and obtained α values of 1.01751 (Cathedral main chamber summer), 1.01965 (Cathedral main chamber winter), and 1.01161 (Gaden main chamber summer). These values all fall within the ranges reported for aerobic oxidation of CH_4 (Grant and

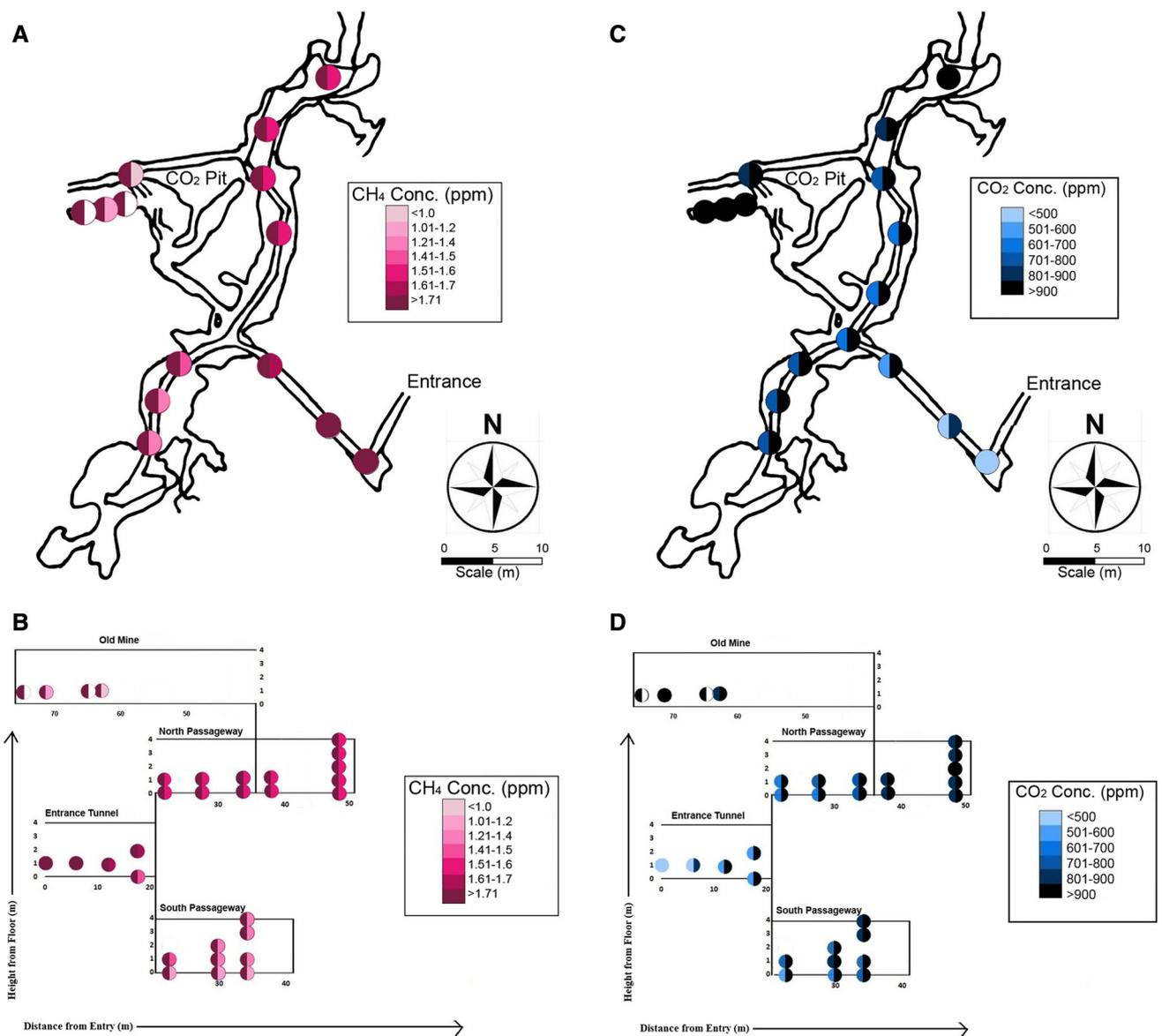


Fig. 5 Birds eye view diagrams showing CO₂ (a) and CH₄ (c) in Gaden Cave at 1 m height from cave floor and schematic diagrams showing CO₂ (b) and CH₄ (d) concentrations in Cathedral Cave. The

left side of the coloured points indicates winter 2014 values. The right side indicates summer 2015 values

Whiticar 2002; Matthey et al. 2013). This analysis further supports that microbial processes at Wellington Caves dominate CH₄ oxidation. No analysis was performed on the Gaden Cave winter data, as all the data are clustered around outside ambient air (Figure S.8), and displayed no evidence of oxidation.

PCA analysis

The results of the two leading components in the PCA are presented in Fig. 6. The PCA generated four principal components (PC), with the first two explaining 84 % of the variability in the dataset. Figure 6 shows that PC1 explains

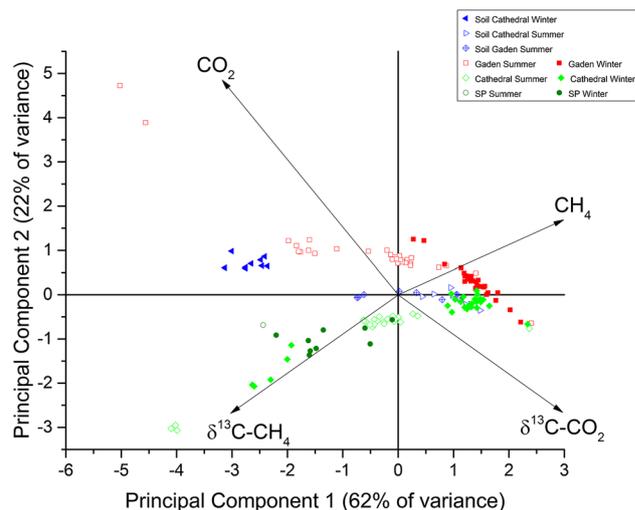
62 % of the variance in the data, with positive scores correlating with high [CH₄] and negative scores with high [CO₂]. PC2 explains 22 % of the variance in the data, with negative scores correlating with heavier isotopic compositions of both CO₂ and CH₄.

Figure 6 shows that that the majority of the data fall between two end-members. One comprises the winter soil samples, which represent a high [CO₂]/low [CH₄], and light ¹³C–CO₂ end-member. The other is a low [CO₂]/high [CH₄] and heavy δ¹³C–CO₂ end-member, which comprises data from Cathedral and Gaden cave entrance samples. We interpret this pattern in the data as representing the two dominant inputs for CH₄ and CO₂ in the cave system: (1)

Table 2 Average, median and standard deviation of [CO₂] and [CH₄] and δ¹³C (‰) values at locations within Cathedral Cave and Gaden Cave in winter/August 2014 and summer/February 2015

	Cathedral Cave				Gaden Cave					
	South Passage		Thunder Cave		North Passageway		South Passageway		Old Mine	
	Conc. (ppm)	δ ¹³ C (‰)	Conc. (ppm)	δ ¹³ C (‰)	Conc. (ppm)	δ ¹³ C (‰)	Conc. (ppm)	δ ¹³ C (‰)	Conc. (ppm)	δ ¹³ C (‰)
Average										
CO ₂	3535 (7784)	-17.58 (-19.18)	1013.09 (1098.40)	-16.39 (-16.37)	761 (5703)	-14.25 (-20.47)	792 (2248)	-14.45 (-18.45)	1796.12 (30,792.01)	-17.64 (-17.08)
CH ₄	0.87 (0.27)	-37.54 (-24.60)	0.83 (0.52)	-33.01 (-22.75)	1.79 (1.52)	-46.96 (-42.50)	1.79 (1.54)	-46.96 (-46.09)	1.77 (0.98)	-46.80 (-35.27)
Median										
CO ₂	1117 (5326)	-16.67 (-19.25)	982.19 (1087)	-16.20 (-16.59)	756 (5808)	-14.27 (-20.32)	840 (2283)	-14.52 (-18.46)	1787 (30,792)	-18.16 (-17.08)
CH ₄	1.08 (<0.50)	-36.08 BDL	0.99 (0.50)	-32.39 (-22.75)	1.79 (1.24)	-46.94 (-41.94)	1.78 (1.24)	-47.02 (-41.94)	1.77 (0.98)	-46.79 (-35.27)
Standard deviation										
CO ₂	4769 (6375)	2.79 (0.73)	94.22 (68.33)	0.67 (0.34)	91 (1008)	0.87 (0.66)	117 (208)	1.08 (0.44)	930 (2390)	2.15 (0.76)
CH ₄	0.12 (0.36)	2.32 (16.29)	0.08 (0.02)	2.43 (0.28)	0.01 (0.08)	0.13 (1.30)	0.01 (0.02)	0.25 (0.52)	0.02 (0.04)	0.26 (0.90)

Summer 2015 values are shown in brackets

**Fig. 6** Principle Components Analysis (PCA) of CO₂ and CH₄ concentrations in Cathedral Cave, Gaden Cave, soil-air and atmospheric-air samples for winter and summer

soil-derived air and ground air entering the cave through fractures and fissures, and (2) atmospheric CO₂, entering the cave from the entrance. This pattern can be explained by cave ventilation, and the PCA suggests that this is the main process controlling CO₂ and CH₄ in the main passages of Cathedral Cave and Gaden Cave. The PCA also suggests a strong seasonality in the soil CO₂ source: in our data the winter soil [CO₂] was highest as this was a period of high rainfall, soil moisture content and increased microbial and root respiration. Also of note in the PCA is that Cathedral Cave and Gaden Cave have slight differences in the trend lines, with higher [CH₄] visible in Gaden Cave compared to Cathedral Cave.

Several samples do not plot on the atmosphere-soil trend line (refer Figs. 6, S1–7), including all samples from South Passage in Cathedral Cave, all summer samples from Cathedral Cave, and two summer samples from Gaden Cave (G13 and G15). Winter samples from Cathedral Cave site 13 at Thunder Cave also plot slightly away from the trend line. These samples are either away from the main stem of the caves and therefore less affected by ventilation, or occur in summer when cave ventilation is lower. All score positively in PC1, and the majority also score positively in PC2, indicative of δ¹³C-CH₄ that is isotopically heavier. The two summer Gaden Cave samples also score positively on PC3 (data not shown), which explains a further 14 % of the variability in the dataset and correlates with high concentrations of isotopically heavy CO₂. The PCA therefore suggests two separate patterns in these samples. At G13 and G15, there is a summer signature which has high CH₄ and CO₂ concentrations and heavy isotopic composition of CH₄. In South Passage, there is high [CO₂] and low [CH₄], where the CH₄ isotopic composition is heavy.

Discussion

Bedrock CO₂ sources

No clear evidence was found of CO₂ sourced from bedrock. Results of IRMS of both thinly bedded ($\delta^{13}\text{C} = 2.9 \pm 0.1 \text{ ‰}$) and massive limestone ($\delta^{13}\text{C} = 1.5 \pm 0.1 \text{ ‰}$) indicate typical bedrock compositions. The isotopic composition of degassed CO₂, comprising a mixture of organic and inorganic sources, would lie between these two end-members and should only be found in Cathedral Cave where dripwater or supersaturated groundwater is present. The cave-air CO₂ isotopic composition lies close to the soil end-member in winter where ventilation is occurring and where degassing rates should be at their peak. This suggests that there is minimal CO₂ sourced from bedrock within the cave-air. One potential source of cave-air CO₂ is degassed CO₂ from supersaturated groundwater, however, $\delta^{13}\text{C}$ -CO₂ values at The Well in Cathedral Cave suggest organic CO₂ sources. The [CO₂] in this part of the cave suggests that any contribution from degassing is negligible.

Soil CO₂ sources

Diffusion of atmospheric air in the surface layer of the soil profile (0–10 cm) at Cathedral Cave appears to be low, but is difficult to detect given the high concentration of biologically produced CO₂ in the upper soil profile (>5000 ppm at 0–10 cm above Cathedral Cave). There is also minimal mixing between the atmosphere and the upper layer of soil due to the low permeability of the soils, which would inhibit the mixing of atmospheric air with soil air. The soil-air CO₂ above Cathedral Cave has a winter $\delta^{13}\text{C}$ -CO₂ source signature value of -22.5 ‰ (95 % CI: -23.2 to -21.9 ‰) (Figure S.2) and a summer $\delta^{13}\text{C}$ -CO₂ value of -18.7 ‰ (95 % CI: -19.8 to -17.7 ‰) (Figure S.1). The soil air above Gaden Cave in summer returned a $\delta^{13}\text{C}$ -CO₂ value of -19.5 ‰ (95 % CI: -19.8 to -19.1 ‰) (Figure S.3). These values correspond to the respiration and breakdown of a predominantly C₃ vegetation source with a C₄ or CAM vegetation contribution. The soils appear to have higher [CO₂] during winter than summer. In semi-arid regions where soils dry out in summer, this is expected.

Mechanisms of CO₂ and CH₄ production, consumption and transport in Gaden and Cathedral caves

Cathedral Cave

Whilst both caves show soil-derived source signatures, there is a seasonally consistent inverse correlation between

CO₂ and CH₄ in Cathedral Cave (Fig. 4) (winter $R^2 = -0.45$; $n = 40$; $p = 0.00$; summer $R^2 = -0.43$; $n = 40$; $p = 0.00$). This trend is also seen in Gaden Cave in summer ($R^2 = 0.61$; $n = 29$; $p = 0.00$) but not in winter ($R^2 = 0.17$; $n = 30$; $p = 0.03$) (Fig. 5). This indicates that there are different pathways contributing to the [CO₂] and [CH₄] observed in each cave during the two seasons. CH₄ in the soils overlying both Cathedral and Gaden caves appears to be both produced and oxidised in situ. In soils above Cathedral Cave this CH₄ is likely to be predominantly produced by acetate fermentation rather than CO₂ reduction, evidenced by the reduced correlation between summer soil-air CO₂ and CH₄ ($R^2 = 0.57$; $n = 7$; $p = 0.05$) as compared to Gaden Cave ($R^2 = 0.98$; $n = 9$; $p = 0.00$). The correlation between winter soil-air CO₂ and CH₄ above Cathedral Cave is also low ($R^2 = 0.11$; $n = 9$; $p = 0.39$). Acetate fermentation is a dominant form of methanogenesis in freshwater environments (Winfrey et al. 1977; Whiticar 1999). The presence of methanogens, however, is evident in soils above both caves as verified by the soil $\delta^{13}\text{C}$ -CH₄ values. These values indicate biological production with subsequent consumption of the lighter C-isotopes. Due to the highly CH₄-depleted air present in soils above Cathedral Cave (concentrations as low as 0.89 ppm in winter), it is possible that soils at depths greater than 40 cm from the soil surface above Cathedral Cave are undergoing low affinity methanotrophy, where high concentrations of CH₄ are produced by methanogens in saturated areas of the soil or cavities above the cave. This CH₄ may then be subsequently oxidised to CO₂ close to the site of production, causing high [CO₂] in the cave below. Anaerobic areas suitable for methanogens to produce high amounts of CH₄ may be caused by high soil moisture retention or water reserves above the cave, as indicated by entry of dripwater into sections of Cathedral Cave.

There is evidence for CH₄ production in summer at The Well due to the increased [CH₄] (1.62 ppm) compared to winter (1.21 ppm) when ventilation should increase [CH₄] to values closer to atmospheric. Production and ebullition of CH₄ is to be expected here due to the anoxic environment of the groundwater. There is also increased [CO₂] in this area during summer (915–1077 ppm (summer); 600–718 ppm (winter)) suggesting that CH₄ is produced here and oxidised to produce CO₂ in situ. This interpretation is, however, limited due to the lack of microbiological analysis of soil samples from within the caves. It is recommended that future studies include the microbiological analysis of soil samples obtained from within the cave as well as overlying soils.

South Passage has a CO₂ source signature that is lighter than the overlying soil source in winter. This could indicate that there has been some fractionation of the CO₂ (¹²C

diffuses faster than ^{13}C) as it travels through pore spaces in soil before entering the cave (Meyer et al. 2014).

Gaden Cave

The near-atmospheric $[\text{CH}_4]$ (shown in Fig. 4a, b) and isotopic ratios throughout Gaden Cave in both winter and summer (when cave ventilation is absent), indicates minimal production or consumption of CH_4 within the cave. This may be due to soil and bedrock properties. Speleothems in the cave have only been observed to have active dripping after very wet conditions (such as in 2010/2011), suggesting that void spaces and fractures in the limestone over Gaden Cave have limited water storage. This could prevent areas for water to accumulate, thus preventing anoxic zones for methanogens to thrive. Reduced soil moisture has been shown to reduce microbial CO_2 production (Fierer et al. 2003).

Mixing plots indicate that the source of CO_2 entering Gaden Cave in winter is organic and soil derived [$\delta^{13}\text{C} = -20.9\text{‰}$ (95 % CI -21.2 to -20.7‰) (Figure S.4)]. The near-atmospheric $[\text{CH}_4]$ found throughout the entire cave system in February suggests that soil CO_2 is only partially contributing to the CO_2 present in the deeper parts of Gaden Cave in summer. Oxidation of CH_4 to CO_2 is evident in the soils above Gaden Cave in summer by the correlation between CH_4 and CO_2 ($R^2 = 0.98$; $n = 9$; $p = 0.00$), however, within the cave this correlation is reduced ($R^2 = 0.61$; $n = 29$; $p = 0.00$). The source of CO_2 in Gaden Cave appears to be a mixture of some contribution of respired CO_2 from plant roots, and CO_2 produced through a glycolic pathway of organic matter decomposition within the bedrock. The cause of the isotopically heavy CO_2 in “Old Mine” in summer is uncertain. However, it is speculated to be caused by either a process that removes isotopically light CO_2 , such as uptake by plant roots, or due to the settling of heavy CO_2 in deep parts of the cave in non-turbulent cave-air environments when ventilation is not occurring.

A study performed by the Australian Radiation Laboratory, the University of Sydney and the University of Auckland provides results of cave-air ^{222}Rn levels in 52 cave systems around Australia (Solomon et al. 1996). These results show that Cathedral Cave and Gaden Cave have annual average ^{222}Rn levels of 330 and 276 Bq m^{-3} , respectively. The highest seasonal average levels in both caves occur in summer, at 436 Bq m^{-3} in Cathedral Cave and 532 Bq m^{-3} in Gaden Cave. The lowest levels in both caves were measured in winter at 124 and 94 Bq m^{-3} in Cathedral and Gaden caves, respectively. Due to these annually and seasonally low levels of ^{222}Rn , it is unlikely that the ionisation of gas molecules by ^{222}Rn decay is a significant factor in the oxidation of CH_4 in these caves.

Monitoring of the density of cave-air ions is suggested as a key area for future research.

The results from this study suggest that the cause of the high $[\text{CO}_2]$ found in the “ CO_2 pit” in Gaden Cave (Osborne 1981) may be due to accumulation and stratification of soil-derived CO_2 in the deeper parts of the cave. Ground-air CO_2 is probably also a contributor as this was shown by Atkinson (1977) to increase in concentration with depth. As the “ CO_2 pit” is separated from Gaden Cave’s main passageways it is not exposed to wind gusts or air turbulence, particularly in summer, when there is a lack of cave-air ventilation. Evidence of CO_2 stratification was found in Cathedral Cave where high concentrations of CO_2 ($>25,000$ ppm in summer) were observed in a crawl-space (up to 1.5 m lower than surrounding ground floor) in South Passage (site SP4). The two crawl-space air samples collected in South Passage have biological $\delta^{13}\text{C}-\text{CO}_2$ signatures of $-21.5 \pm 0.6\text{‰}$ in winter and $-19.4 \pm 0.6\text{‰}$ in summer. Both of the SP4 sample values, whilst extremely high in CO_2 , displayed an isotopic signature that appears to be disproportionately heavier than the signatures of the air samples surrounding it. Isotopic studies of air samples taken directly from within the “ CO_2 pit” would be needed to allow for a clearer identification of the source.

Implications for speleothem/paleoclimatic studies

Our results suggest that elevated $[\text{CO}_2]_{\text{cave}}$ in a semi-arid climate is predominantly soil and vegetation derived, even at a location where evaporation greatly exceeds rainfall, soil CO_2 production is water limited and there is a source of CO_2 from degassing of supersaturated groundwater. Our unpublished cave dripwater data demonstrates a range of Ca^{2+} concentrations of 2.2 ± 1.0 mmol/l; therefore $[\text{CO}_2]_{\text{cave}}$ over 2000 ppm could inhibit speleothem growth (Baker and Genty 1998), especially at locations that are further from the entrance and in less ventilated passages and chambers. For example, the highest $[\text{CO}_2]_{\text{cave}}$ was measured in summer, when soil-air $[\text{CO}_2]$ was lowest. The highest soil-air $[\text{CO}_2]$ in summer was 2086 and 1190 ppm above Gaden and Cathedral caves, respectively. At the same time, $[\text{CO}_2]_{\text{cave}}$ reached 28,780 and 25,542 ppm in Gaden and Cathedral caves, sufficient to inhibit speleothem growth for specimens with dripwater Ca^{2+} concentrations lower than ~ 2.2 mmol/l. This raises the possibility of sampling speleothems on a transect of $[\text{CO}_2]_{\text{cave}}$, and investigating the timing of speleothem growth as a record of soil CO_2 productivity. Researchers might also choose to sample paired specimens from less (more) ventilated locations and higher (lower) cave-air CO_2 to attempt to contrast and quantify the effects of rapid degassing and associated fractionation of speleothem proxies such as $\delta^{18}\text{O}$.

Implications for CH₄ and CO₂ concentrations in other caves

Cave morphology appears to be a good predictor of [CO₂]_{cave}, as proposed by James et al. (2015). Caves with multiple narrow, descending, dead-end pathways leading away from main entrance passages are more likely to have high [CO₂], even in winter. Higher [CO₂] is expected to occur with increased depth underground in unventilated areas. Steep dips and large holes in the cave floor of isolated passages enable the accumulation and stratification of large amounts of CO₂ when not affected by cave ventilation.

Globally it is possible that many caves located in arid or semi-arid areas will be experiencing low CH₄ production or consumption. This is particularly likely when soil moisture is low and water reserves are not present, resulting in a lack of microbiologically derived CH₄ production. Small soil particle size and/or sections of dissolved limestone above caves appear to counteract this effect by allowing for the retention of water in soils or voids above the cave systems, providing methanogens and methanotrophs a suitable environment in low-rainfall areas. Saturated fissures in the epikarst appear to be an area where methanogenesis can take place with little or no oxidation. Caves in wetter climates are likely to produce high [CH₄] in overlying soils or bedrock, which may then be oxidised into high concentrations of CO₂ by methanotrophs. Further research could focus on the analyses of ground-air to determine whether it is possible to differentiate between CO₂ or CH₄ produced in the soil and that which is produced in the bedrock, and whether these differences are visible in cave-air.

In other cave systems, low soil moisture, soil pH or other soil and bedrock properties may reduce the amount of methanogenesis and methanotrophy in the cave and overlying soil. In this case, atmospheric CH₄ remains unmixed with CH₄-depleted air and near-atmospheric concentrations and δ¹³C values are observed throughout the cave. This may be the case with Gaden Cave.

Implications for the global carbon budget

The results of this research also have important implications for global carbon budget estimates. It is evident that all karst environments differ in [CO₂] according to morphology, climate and soil influences. When assessing the impact of karst environments in carbon accounting, it will be important to consider the process of carbon exchange by CH₄ oxidation to CO₂. Ascertaining the amount of carbon exchange will aid in the quantification of the contribution of CO₂ by sources that are unrelated to the oxidation of atmospheric CH₄. This will allow for the determination of the true contribution of CO₂ equivalence from the karst landscape.

Summary and conclusions

The δ¹³C–CO₂ of cave-air in Gaden and Cathedral caves suggest that even in arid and water-limited countries, cave-air CO₂ has a predominantly biological soil source, with [CO₂]_{cave} and [CH₄]_{cave} largely determined by cave morphology and soil and bedrock properties. Climate variability has the potential to change temperature, rainfall and evaporation rates, which will affect a large number of environmental factors. These include soil moisture, pH, temperature, vegetation cover (amount and type) and the soil microbiological community. Such climatic changes may have additional influences on soil [CO₂] and δ¹³C–CO₂ values, which must be better constrained to assess the impacts on climatic signals recorded in speleothems.

Cave-air CH₄ analyses from Cathedral and Gaden caves indicate that the ability of a cave to act as a net sink for CH₄ varies widely. Shallow well-ventilated caves, like Gaden Cave, act as CH₄ sinks in summer. In winter, the continuous movement of external atmospheric air throughout such caves replenishes the cave air at a rate such that CH₄ oxidation is not discernible. In contrast, larger deeper caves, such as Cathedral Cave, act as a CH₄ sink throughout the year. The rate of CH₄ oxidation within this type of cave remains constant throughout the year, however, the concentration of CH₄ varies throughout the seasons as density-driven cave ventilation oscillates between summer and winter states. Further research into [CO₂] and [CH₄] in semi-arid karst and other unstudied karst environments, and their determining variables, is required to realise the true extent of the ability of karst landscapes to act as net sinks for CH₄. This will affect how karst environments are considered in global carbon budget assessments.

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