

Geochemical signatures of surface environmental changes in two Australian caves: fire signals and organic carbon archives

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# GEOCHEMICAL SIGNATURES OF SURFACE ENVIRONMENTAL CHANGES IN TWO AUSTRALIAN CAVES:

## FIRE SIGNALS AND ORGANIC CARBON ARCHIVES

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# Thesis/Dissertation Sheet

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#### Abstract 350 words maximum: (PLEASE TYPE)

Wildfire and climate changes have been broadly reported to generate accumulated effects on ecosystem flora and fauna. Although enormous of studies have been conducted to illustrate these surface events, geochemical evolutions into the subsurface system are difficult to quantify. This thesis aims to combine the isotope analysis, hydrograph analysis and geochemical analysis to provide insights on the karst hydrology and paleoenvironment reconstructions. In short term, wildfire has been reported to modify burnt surface properties including soil hydrophilicity, and nutrient generation as ashes dissolve. A severe 10 m x 10 m experimental fire was conducted above the Wildman's Cave in Wombeyan Caves, New South Wales, Australia in May 2016. After the fire, loss of water was observed associating with the decreased drip water  $\delta^{18}$ O value by 6.3 per mille. A lag corresponding to the depth of each site was discovered. Both increased preferential flows and decreased capillary flows were reflected via hydrograph analysis in respect of individual discharge events. Fire-derived volatilization of the soil and ash elements occurred. Post-fire monitoring shows a recovery trend based on the stable water isotopes to the initial values in the following half year.

Over longer timescales, organic matter in cave stalagmites has the potential to assist in paleoenvironment reconstruction. In this thesis, an innovative  $\delta^{I3}$ C analysis method was proposed for entrapped organic matter in stalagmites. Acquired organic  $\delta^{I3}$ C from Easter Cave Flowstone 1 (ESFI) were compared with the calcite  $\delta^{I3}$ C and  $\delta^{I8}$ O. Our results show that this proposed method is reliable for the analysis of organic matter (OM) under constant conditions, with a low average standard deviation (0.2 per mille) and low sample consumption (25 mg). The fluctuations on the time series of calcite  $\delta^{I3}$ C for the ESFI were recognised to be the results from fractionation, without climate and surface vegetation changes factors.

In short term, it reveals the story occurring after fire, with increased preferential flows and decreased capillary flows. In longer timescales, an innovative insight onto the carbon isotope fractionation mechanism and paleoenvironment reconstruction is created. These results have improved current understandings to the importance of surface and subsurface systems.

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June, 2018

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#### Abstract

Wildfire and climate changes have been broadly reported to generate accumulated effects on ecosystem flora and fauna. Although enormous of studies have been conducted to illustrate these surface events, geochemical evolutions into the subsurface system are difficult to quantify. This thesis aims to combine the isotope analysis, hydrograph analysis and geochemical analysis to provide insights on the karst hydrology and paleoenvironment reconstructions.

In short term, wildfire has been reported to modify the burnt surface properties including soil hydrophilicity, and nutrient generation as ashes dissolve. A severe 10 m x 10 m experimental fire was conducted above the Wildman's Cave in Wombeyan Caves, New South Wales, Australia in May 2016. After the fire, loss of water was observed associating with the decreased drip water  $\delta^{18}$ O value by 6.3 per mille. A lag corresponding to the depth of each site was discovered. Both increased preferential flows and decreased capillary flows were reflected via hydrograph analysis in respect of individual discharge events. Fire-derived volatilization of the soil and ash elements occurred. Bedrock-derived elements decreased due to decreased dissolution. Post-fire monitoring shows a recovery trend based on the stable water isotopes to the initial values in the following half year.

Over longer timescales, organic matter in cave stalagmites has the potential to assist in paleoenvironment reconstruction. In this thesis, an innovative  $\delta^{13}$ C analysis method was proposed for entrapped organic matter in stalagmites. Acquired organic  $\delta^{13}$ C from Easter Cave Flowstone 1 (ESF1) were compared with the calcite  $\delta^{13}$ C and  $\delta^{18}$ O. Our

results show that this proposed method is reliable for the analysis of organic matter (OM) under constant conditions, with a low average standard deviation (0.2 per mille) and low sample consumption (25 mg). The fluctuations on the time series of calcite  $\delta^{13}$ C for the ESF1 were recognised to be the results from fractionation, without climate and surface vegetation changes factors.

As a consequence, in short term, it reveals the story occurring in the soil, with increased preferential flows and decreased capillary flows after fire. In longer timescales, an innovative insight onto the carbon isotope fractionation mechanism and paleoenvironment reconstruction is created. These results have improved current understandings to the importance of surface environments and linked it to the subsurface system.

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#### CHAPTER 1 Introduction

An enormous number of studies have been conducted on the accumulated effects of wildfires and climate change on ecosystems, flora and fauna (Arno et al., 1977; Bod íet al., 2014; Bonacci et al., 2008; Forchhammer and Post, 2004; Kullman, 2007; Walther et al., 2002). However, there have been few investigations into quantifying the geochemical evolutions of subsurface systems affected by fire due to the many variables existing within such complex circumstances.

Basically, in the karst system, two types of fires could occur: prescribed (controlled) fires and wildfires. Prescribed fires are utilised to burn naturally accumulated fuels to reduce the extent and severity of wildfires, or to facilitate the germination and growth of desired flora species. Wildfires normally occur when there are an adequate fuel load and dry conditions, and are generally severe. Due to the heterogeneous spatial distribution of forest, naturally burnt areas are usually chaotically distributed, forming a mosaic of lightly- and heavily-burned areas (Rab, 1996). Fire-induced changes to soil properties can be short-term, long-term or even permanent, and are influenced by the type of soil, the severity and frequency of fires, and post-fire climatic conditions (Certini, 2005). Post-fire influences on fauna, especially from ash (Khanna et al., 1994; Santín et al., 2015) and plant diversity (Safford and Harrison, 2004; Uys et al., 2004) have been widely investigated. Yet, impacts on the subsurface environment have received little attention despite the importance of hydrology and nutrient availability to ecosystem recovery.

A different method of investigation is the isotopic analysis of speleothems, which can help reconstruct past environmental and climatic conditions. The  $\delta^{13}$ C values of calcite in speleothems are recognised to be directly related to the primary sources of carbon encountered by cave seepage water. For  $\delta^{13}$ C, these sources include soil CO<sub>2</sub>, atmospheric CO<sub>2</sub> and carbonate bedrock dissolution (Baker et al., 2016). Vegetation is one major factor influencing speleothem  $\delta^{13}$ C values, with soil CO<sub>2</sub> from root respiration and microbial oxidation of soil organic matter both being derived from vegetation (Blyth et al., 2016). Through a combination of oxygen and carbon isotopes, isotope analysis has demonstrated feasibility in supplying information on vegetation (Harmon et al., 2004) and local climate (Hellstrom and McCulloch, 2000). One specific interesting case in the development of carbon isotope analysis involves isotope signals based on the C3 or C4 photosynthetic pathway. The C3 and C4 plants produce large differences in  $\delta^{13}$ C values (Smith and Epstein, 1971; Deines, 1980; Vogel, 1983; Dorale et al., 1992). Soil CO<sub>2</sub> from C3 plants have  $\delta^{13}$ C values approximately -23‰, whereas CO<sub>2</sub> from C4 plants approximately -9‰ (Fritz and Clark, 1997). C3 plants include most trees, herbs, shrubs and cool-season grasses, while C4 plants are typically more common in tropical and temperate grasslands. Carbon isotope signals have the potential to reveal vegetation biomass ratios over time (Dorale et al., 1992, 1998; Holmgren et al., 1995).

# 1.1 Hydrology Responses to Surface Environmental Change in Karst System

Variation in drip hydrology can potentially reflect changes in surface environments. In karst systems, karstified carbonate rock is heterogeneous, highly fractured and permeable, such that subsurface water flow develops. There are three levels of porosity defined for karstified carbonate rocks (Ford & Williams, 2007). Primary porosity is associated with inter-granular pore spaces, secondary porosity is associated with inter-granular pore spaces, secondary porosity is associated with certain porosity is associated with solution-enhanced conduits.

In karst systems, water movement is observed to occur through flow paths and porous structures. These subsurface water flows can be rapid through connected conduits and caves, acting like underground rivers. In contrast, matrix flows occur via primary porosity and are relatively slow. However, due to the complex variables in subsurface systems, it is difficult to measure subsurface water flow. One practical method to determine the flow paths recharging one stalagmite is through drip discharge monitoring. The first drip hydrology studies were conducted by Pitty (1966, 1968), who manually recorded seasonal variations in drip chemistry at Pooles Cavern, Derbyshire, UK, over one year. Similar studies were conducted by Gunn (1974) in New Zealand caves, who combined manual measurements with tipping bucket gauges to establish the basics of cave drip hydrology.

Basic hydrological models of unsaturated zone recharge with flow rate fluctuations were first introduced in 1987 (Smart, 1987). Acoustic automated drip loggers have only

recently been utilised in cave hydrology studies, and have proven convenient and efficient in observing cave discharge. They can also monitor greater temporal scales than direct observation methods (Collister and Mattey, 2005). Markowska et al. (2015) investigated the influences on hydrographs from karst architectures with acoustic drop loggers, principal component analysis (PCA) and agglomerative hierarchal clustering (AHC). They proposed a coupled conceptual and box hydrological flow model to understand the depth-discharge relationship. Additionally, the effects of phreatophyte evapotranspiration were reported, revealing that tree water use can be observed in cave recharge patterns (Coleborn et al., 2016a).

Measurement of the water isotopes in drips and meteoric precipitation has helped establish the links between surface and subsurface systems. Quantitative data derived from cave recharge events can complement conventional observations, which have only been conducted over a relatively short period of geological time. Studies of hydrogen and oxygen isotopes in cave drips were initially conducted in 1985 (Yonge et al., 1985). Alison et al. (1983) built models of soil  $\delta^{18}$ O with comparison with the meteoric water line and of local recharge. Then, in 1985, they estimated evaporative water loss using enrichment in deuterium (Allison and Barnes, 1985). Water isotopes are related to the properties of precipitation (Jouzel et al., 2000) and have been used to distinguish between groundwater and surface water (Sophocleous, 2002), as tracers of moisture sources, and to fingerprint catchment residence times and flow pathways (Tian et al., 2007). While multiple factors potentially influence the  $\delta^{18}$ O record in speleothems, adequate evidence has been provided to give it a primary role in speleothem science (Lachniet, 2009). In semi-arid environments, the connection of precipitation with soil water isotopes has been well documented (Brooks et al., 2010; Mathieu and Bariac, 1996; Stewart et al., 2001). Meteoric precipitation infiltrating the soil leaves an isotopic signatures in the soil water. Successive evaporation occurring on the surface leads to isotopic enrichment of soil water isotopes. However, when the soil contains a low volumetric water content, large infiltration events can exceed the soil moisture deficit and recharge underground as a mixture of negligible 'old' isotopicallyenriched soil water and new input.

#### 1.2 Inorganic Geochemistry from Ash to Cave

Cave drip waters are able to capture surface variations via changes in recharge geochemistry. These changes are preserved in speleothems. This allows their geochemical and physical properties to be decoded to obtain paleoenvironment and climatic information. Chemical elements, including magnesium, strontium, barium, uranium and phosphorus, have been used in paleoclimate studies at different time scales, providing valuable information such as seasonality, paleoaridity and paleomonsoon activity (e.g. Verheyden et al., 2000; Huang et al., 2001; Treble et al., 2003; Fleitmann et al., 2003; McMillan et al., 2005; Treble et al., 2016). In contrast, there are only a few studies available to understand the influence of intense surface changes such as geochemistry signals brought about by wildfire (Nagra et al., 2016; Coleborn et al., 2018). One recent study conducted by Coleborn et al. (2018) has reported the geochemistry influences using the drip water after a moderate-intensity wildfire. However, an approach that looks into the severe fire is still absent currently.

Soil properties, as well as heat and ash production, are the most effected by changes exerted by fire on soils (Certini, 2013). Different fuels, including biomass, necromass and other soil organic matter, are transformed by fire into ash and other products with modified chemical and physical properties. Layers of ash and residues from this transformation cover the surface and produce ecological, hydrological and geomorphological effects. Woods and Balfour (2010) reported fire-derived soil infiltration and erosion rates. Ash is highly soluble and can also be incorporated to soil by downward migration (Pereira et al., 2013). Raison (1979) demonstrated how the physical and chemical properties of soil are modified following the incorporation of ash. Ash also influences soil microorganisms and facilitates vegetation germination and growth, though much of this production might be finished within days or weeks after a fire (Cerdà and Doerr, 2008; Pereira et al., 2013).

Fire spreads rapidly, yielding a mixture of charred particles and mineral ashes (Ormeño et al., 2009; Saura-Mas et al., 2009). During heating, thermal degradation leads to elemental volatilizations. As temperatures increase from 350 °C to 450–500 °C, organic components are largely consumed (Quill et al., 2010; Hogue and Inglett, 2012; Balfour and Woods, 2013), producing lighter ash colours (Lentile et al., 2009; Hogue and Inglett, 2012; Pereira et al., 2013). At this stage, the inorganic components of ash include calcium, magnesium, potassium, silicon, and in lower proportions, phosphorous, sodium and sulphur, and metals including aluminium, iron, manganese and zinc (Pereira and Úbeda, 2010; Gabet and Bookter, 2011). Over 500 °C, some proportions of these elements decrease due to the lower volatilization temperature (Hogue and Inglett, 2012). At temperatures higher than 1400 °C, the ash produced

becomes chemically similar to the products of thermal decomposition reactions (Balfour and Woods, 2013).

#### 1.3 Organic Carbon Archives in Speleothem

Organic matter is contained in trace amounts in fossilised calcareous skeletons, fluid inclusions in minerals, carbonate cements, carbonate particles and speleothems. Although it is the minor component in speleothems, organic materials serve as potential contributors to carbon isotope archives and are also the source of stalagmite colour (Hill and Forti, 1997). Changes in the  $\delta^{13}$ C of stalagmite calcite have been associated with the overlying vegetation regime being dominated by C3 and C4 plants, as proposed above. However, significant fluctuation in  $\delta^{13}$ C has also been observed at sites without climate-derived changes in the ratio of C4 to C3 plants (Baker et al., 1997a; Genty et al., 2003; McDermott, 2004). The function of  $\delta^{13}$ C is still poorly understood, and improvement in organic carbon isotopic analysis is essential to address this.

There are several important sources for the biogeochemical product that is speleothem organic  $\delta^{13}$ C. Primary organic matter may be captured if water soluble, during flow over the surface containing the surface soil and vegetation. Biochemical processes occur through the transfer process and generate secondary organic matter. Rates of biodegradation can be influenced by climate, gas exchange, atmospheric conditions and hydrological properties. Both primary and secondary organic matter are eventually preserved in stalagmites, forming paleo-records (Blyth et al., 2016). The compositions of organic matter in stalagmites are diverse and poorly understood.

Pollen and fungal spores or other airborne volatile compounds can be absorbed from airborne materials. Only limited studies have been conducted on this source of organic matter (Blyth et al., 2008; Brook et al., 2010; Walochnik and Mulec, 2015). However, there is evidence that formations near cave entrances or chimneys can capture airborne loads, with the amount negatively correlated with the distance inside the cave (Peterson, 1976). Biomarkers can also include animal and insect excreta deposited inside the cave. The mechanisms of their biodegradation during the stalagmite formation process are yet to be investigated.

The majority of organic matter is transported from the surface soil through the process mentioned above. The leaching of organic matter from soil is controlled by the partitioning of the dissolution process, which is influenced by soil hydrophilicity, flow, pH, etc., and external climate factors (Blyth et al., 2016). For instance, plant lipids were found to play a vital role in plant uptake of lipophilic contaminants from soils such as aldrin, dieldrin, heptachlor and heptachlorepoxide (Chiou et al., 2001), which are generated from the external surface and the cave ecosystem (Cacchio et al., 2004), hence being potential fingerprints of surface vegetation records.

Carbon cycles, or the turnover of organic matter in karst systems, varies in timescale. Michalzik et al. (2003) indicate that there is continual metabolic production of easily leached humic materials, while carbon concentrations and fluxes at greater depths are controlled by the sorption process. Short-term decay occurs within one year, but steady states are only reached after hundreds-to-thousands of years. Surface soil organics generally take 1–10 years to be incorporated into speleothems (Genty et al., 1998). The length varies due to different degradation and transportation processes and the diverse chemical structures of organic matter (Blyth et al., 2016). Different responses to climate take place depending on the types of organic matter. An additional proportion of organics can be attributed to the dissolution of limestone in flow paths, particularly that with biofilm on its surface (Macalady et al., 2006). However, the understanding of these factors is far from complete. Speleothem organic matter has received too little study to supply a comprehensive conclusion on fractionation.

#### 1.4 Aims of the Thesis

This study aims to further our understanding of underground geochemical signals of surface environment variations, including drip hydrology (Chapter 2), inorganic chemistry (Chapter 2) and organic carbon and calcite isotopes (Chapter 3). Specifically, the following hypotheses were tested.

- Underground cave recharge will respond to intense fire events in karst systems through the influences of surface properties with affected flow paths, which will be reflected in hydrographs.
- Ash produced by fire can be captured by the subsurface system, and changes in specific elements help quantify the volatilisation of nutrient elements at high temperatures.

- 3. The surface vegetation distribution changes over time, directly influencing the organic matter co-precipitated in stalagmites, which can be determined by the analysis of organic carbon isotopes in stalagmites.
- 4. Combinations of calcite carbon and oxygen isotopes with the organic carbon isotopes in speleothem can help reconstruct paleoclimate.

Chapter 2 explores the impact of experimental fires on a shallow cave system in Wombeyan Caves, New South Wales, especially the influence on drip hydrology, water isotopes and inorganic chemistry. Hydrograph analysis describes how hydrological changes to the overlying environment can be reflected through drip hydrology, and a fire model describes the effects of fire on the surface environment over a one-year scale, especially on the preferential flows and capillary flows found in soil and karst. Chapter 3 describes the development of a micro-analytical method to assess organic carbon isotopes in stalagmites comparing to traditional calcite carbon and oxygen isotopes to determine their roles in reconstructing the paleoclimate. The fractionation theory broadly describes the reconstruction of paleoenvironment using carbon isotopes among half of the Holocene period.

The motivation for this thesis came partly from my own interests as an instrumental analyst and geochemist. The knowledge gaps, existing in the study of surface influences on subsurface changes, demand multidisciplinary study. There used to be little understanding of how fires modify the subsurface and how organic carbon helps in paleoclimatic reconstructions. This research may enable better fire management in karst environments and reveal fire records in speleothems.

# CHAPTER 2 Hydrological and Geochemical Responses to Fire in a Shallow Cave System

#### Abstract

The influence of wildfire on surface soil and hydrology has been widely investigated, while its impact on the karst vadose zone is still poorly understood. A severe 10 m x 10 m experimental fire was conducted above the shallow Wildman's Cave in Wombeyan Caves, New South Wales, Australia in May 2016. Continuous sampling of water stable isotopes, inorganic geochemistry and drip rates were conducted from Dec 2014 to May 2017. After the fire, discharge patterns were significantly altered, which is interpreted as the result of increased preferential flows and decreased capillary flows in the soil. Post-fire drip water  $\delta^{18}$ O decreased by 6.3‰ in the first month relative to the pre-fire isotopic composition. Post-fire monitoring showed a recovery in drip water  $\delta^{18}$ O in the following half year. After that, enriched isotopes in the burnt area indicated an decreased albedo from changed soil property. Bedrock-related elements (calcium, magnesium, strontium) decreased rapidly after the fire due to reduced limestone dissolution time and potentially reduced soil CO2. Soil-related elements and ashderived elements both decreased after the fire due to volatilization at high temperatures.

#### 2.1 Introduction

The lack of quantification of the impact of fire events on sub-surface systems, especially in karst environments, limits our understanding of the effects of wildfire and the application of prescribed burning. Only a few recent studies examining long-term fire impacts on karst systems have been published (Coleborn et al., 2016b; Nagra et al., 2016; Treble et al., 2016; Coleborn et al., 2018). Coleborn et al. (2016) compared soil CO<sub>2</sub> concentrations, temperatures and moisture between burnt and unburnt soils at an algine site in Australia. For the first five years, soil respiration was depressed in the burnt forested site and less biomass was reported relative to the unburnt forested site. No significant difference could be seen in the ten-year post-fire soil groups relative to the unburnt control regions. Treble et al. (2016) reported nine-year data on drip water geochemistry, suggesting that the greatest impact of fire is associated with long-term decreases in sulfur concentration, due to post-fire accumulation of this ash-derived nutrient in biomass. Aside from ash signals, higher  $\delta^{18}$ O and chloride levels were associated with increased evapotranspiration in the soil and shallow vadose zone after a wildfire (Nagra et al., 2016). Compared with the bi-decadal time period needed for post-fire habitat and fuel recovery in forest ecosystems (Haslem et al., 2011), soil CO<sub>2</sub> recovery (Coleborn et al., 2016b), and cave drip water isotope, chlorine and sulfur residuals (Treble et al., 2016) are notable in having a multi-year temporal response.

The impact of wildfire on soil structures has been widely investigated (Fernandez et al., 1999; Pausas et al., 2009; Scott and Van Wyk, 1990). Scott and Van Wyk (1990) reported reduced soil wettability following wildfire. Any influence on soil structures

would subsequently affect preferential flow along macropores (Beven and Germann, 1982; Ghodrati and Jury, 1992). Cave drip discharge is potentially affected by physical characters such as soil capillarity (Fredlund and Rahardjo, 1993) and preferential flows (Šimůnek et al., 2003). Fire can induce a more hydrophobic soil structure, which can increase post-fire runoff and erosion in burnt areas (Huffman et al., 2001), leading to significant increases in soil loss, and total and quick flow volumes in streams. Fernandez et al. (1999) observed that ephemeral streams have increased soil organic matter lability, while constant ones have increased organic matter stabilisation. Assessment of mineralisation showed that the surface layer had not recovered more than two years after a fire.

Any surface fire signal transmitted to a cave will pass through the vadose zone—the unsaturated area between the surface and the groundwater level. In karst environments, the vadose zone hosts the flow pathways and water storage that feed cave drip water, and is where the major processes of bedrock dissolution, mixing and dilution occur (Wood, 1985). Other hydrochemical and biogeochemical processes along these pathways have also been identified, such as prior calcite precipitation (Fairchild and Treble, 2009), evaporation (Cuthbert et al., 2014) and nutrient and water uptake by vegetation (Treble et al., 2016). The incorporation of karst hydrology, water isotope tracers and trace element mobility are vital in understanding the complexity of flow pathways (Kogovšek, 2010).

Precipitation percolates through the vadose zone into caves as either *diffuse* or *conduit flows* (Atkinson, 1977). Diffuse flows normally occur at centimetre scales, whilst

conduit flows occur via larger scale fractures or conduits (Pape et al., 2010). Relatively lower flow rates and more stable geochemical properties are associated with diffuse flows, whilst higher flow rates and more variable chemistry characterise conduit flows. Basic hydrological models of unsaturated zone recharge that include flow rate fluctuations were first developed in 1987 (Smart, 1987). Baker et al. (1997b) identified that antecedent precipitation conditions were an important control of the individual hydrological patterns linked to the vadose zone's water storage capacity. Acoustic drip counting was recently introduced as an alternative means of drip recording. Such methods can count falling drips, even during transient events, and record small fluctuations for up to 1–2 years (Collister and Mattey, 2008). In specific regions, the stable isotopes of water ( $\delta^2$ H and  $\delta^{18}$ O) can reveal links between climatic factors and flow pathways (Soulsby et al., 2000). This current research aims to do this for a karst vadose zone.

Trace element concentrations in cave drips reflect changes in external forcing (Tremaine and Froelich, 2013) and changes occurring along flow pathways. Multiple lines of evidence are typically crucial in qualitative models of karst vadose zone hydrological behaviour. Drip water trace element concentrations can be variable even within the same cave chamber (Fairchild and Treble, 2009). Theoretically, the ratio between Mg or Sr and Ca is recognised to be an important factor in karst hydrology (Ternan, 1972). Nutrient elements vary in response to differences in vegetation distribution (Wassenburg et al., 2012), the formation of bedrocks (Immenhauser et al., 2007) as well as the residual duration of each recharge site (Huang and Fairchild, 2001). Hartland et al. (2012) reported a correlation between natural organic matter-

transported metals and climatic signals. The quantity of soil organic matter can also be indicated by shifts in the metal ratios of cave drip discharges. Nagra et al. (2016) compared a burnt cave with a nearby control cave. Biomass-sourced, ash-derived elements, together with dissolved bedrock elements, were both reported as a fire signature.

In this research, we aimed to identify the impact of an experimental fire on a shallow karst vadose zone. The fire was deliberately lit above a cave in order to monitor its impact under controlled conditions. We analysed the composition of cave drip water over 2.5 years (Dec 2014 – May 2017) in Wombeyan Cave, a shallow cave system in NSW, Australia. Our monitoring started 1.5 years prior to the fire and continued for one year afterwards. Thus, unlike the previous studies by Nagra et al. (2016) and Treble et al. (2016), which contain only post-fire data, this study also includes pre-fire data to serve as a baseline with which post-fire data may be compared. This greatly assists in our understanding and attempts to quantify the impacts of fire on karst systems. This is the first research to directly compare pre- and post-fire hydrogeochemical components and drip discharges in a shallow karst vadose zone after the severe fire. Comparisons of pre- and post-fire discharge patterns and water stable isotope concentrations were made to demonstrate changes to the local vadose zone's hydrology, while inorganic geochemical changes were also analysed as supplementary evidence.

#### 2.2 Site Description

The Wombeyan Caves Karst Conservation Reserve (34° 18' S, 149° 58' E) is located in the south-eastern part of New South Wales, Australia, on the western edge of the Sydney Basin, in a plateau of the Great Dividing Range, surrounded by agricultural areas (Figure 2.1). Wombeyan Limestone within the Bungonia Group is completely surrounded by effusive igneous rocks of the Lower-Middle Devonian Bindook Porphyry Complex (Brunker and Offenberg, 1970; Osborne, 1984). It is Silurian, highly fractured and almost entirely marbleised carbonate with no primary porosity remaining (Osborne, 1993). Therefore, only fracture and conduit flows could occur in the karst. Typically, thin soils (< 4 cm depth) above the caves contain gravel, marble fragments, red silty clay and dark humic matter (McDonald and Drysdale, 2007).

The mean annual temperature at Wombeyan Caves ranges a maximum of 26.0 °C in January to a minimum of 0.6 °C in July. Long-term precipitation has an annual median of 684.7 mm (1942-2017, from BOM 063093), with summer precipitation exceeding winter precipitation by 47%. More recently, during December 2014 to May 2017, winter monthly precipitation (104 mm) exceeded that of summer by 32% (79 mm).

Wildman's Cave (W456) is a small and shallow cave near the top of a ridge above Mares Forest Creek Gorge. The cave has a narrow pothole-type entrance and 42 m of reasonably decorated passage (Ellis and Society, 2004). The single large chamber is approximately 15 m long and 6 m wide, with less than 1 m of soil and bedrock overlying the cave.



Figure 2.1 Location of Wildman's Cave in Wombeyan Caves, New South Wales, Australia (a), 3D diagram of the study site (b.), exposed Wombeyan limestone (c.), and burnt area (d.). Map Photo (a): Google, DigitalGlobe. Photos (c and d): Andy Baker.

#### 2.3 Method

#### 2.3.1 Experimental Fire

An experimental fire 10 m x 10 m in area was conducted above Wildman's Cave on 27<sup>th</sup> May 2016. Given the experimental nature of the fire, additional fuel (branches and leaf litter collected adjacent to the site) was placed within the burn area to ensure that a moderately intensive burn was achieved. Within the 10 m x 10 m burn area, shrubs and groundcovers dominated the site. There were no mature trees within the site at the time of the fire. The highest recorded temperature was 929 °C in the middle of the burn area at 12 cm depth (see SUPPLEMENTARY FIGURE 1.1. TEMPERATURE RECORDS FROM 26<sup>TH</sup> MAY TO 1<sup>ST</sup> JUNE 2016. THE PROBE WAS BURIED 12 CM BELOW THE SURFACE.) recorded using a thermocouple temperature recorder TCTemp1000 (Thermoworks<sup>TM</sup>). The fire intensity was severe (Keeley, 2009), with canopy cover left intact but the surface litter largely consumed and thick white ash layers generated at hotspots to a depth of several centimetres. The fire was started mid-morning, and visible flame lasted approximately 45 mins.

#### 2.3.2 Location of Monitoring Sites within the Cave

Eleven dripping stalactites in Wildman's Cave were included in this study to monitor the geochemical and hydrological variations associated with the burn (see Figure 2.1 b). In the corner of the pothole-type entrance, a straw stalactite (Site 01) was utilised as a control, as its inferred flow-path was not directly affected by the experimental fire (see Figure 2.1 b). A large cluster of soda-straws separated the entrance from the inner chamber, and provided drips for Sites 02 to 05. Sites 06 to 11 were located within the
large chamber, while Sites 02 to 06 were deeper than the others and had thicker roof layers.

# 2.3.3 In-cave Collection

In-cave monitoring started in Dec 2014 and ended in May 2017. Water samples were collected at approximately two-month intervals throughout the research program, with sample volumes limited by the infrequent occurrence of recharge events. Two drip water sampling methods were employed. Firstly, drip water samples were collected by leaving bottles in the cave for approximately two months. Wide-mouth 120 ml HDPE sampling bottles were placed under each dripping site, with a plastic funnel containing an acoustic data logger placed inside. Each of these bottles used for cumulative drip water sampling contained 2 mm paraffin oil to prevent evaporation or exchange with the atmosphere. Secondly, opportunistic sampling was conducted during the bi-monthly sampling campaigns, provided recharge was occurring. These drip waters were sampled directly into 250 ml wide-mouth HDPE sampling bottles placed directly under the drip sites overnight. The collection of these campaign samples was conducted without funnels or drip loggers to minimise contamination from the surrounding environment. Reference groundwater samples were collected using a local borehole in Wombeyan Caves campground using 250 ml wide-mouth HDPE containers.

#### 2.3.4 Precipitation Collection

Precipitation stable water isotopes were collected from Mount Werong (34° 04' S, 149° 55' E), which was the nearest precipitation isotope collection station to Wildman's

Cave (~30 km to the northeast). Precipitation samples were collected on an event basis using a sealed HDPE bottle with a plastic funnel. The design was based on the method of Gröning et al. (2012) in order to prevent evaporation of the sample or exchange with the atmosphere. The rainfall-related dataset used here was from August 2014 to May 2017, and is an extension of a time series previously published by Hughes and Crawford (2013) and further described by Crawford et al. (2013).

# 2.3.5 Drip Hydrology

Hydrology records were comprised of drip rates from stalactites within the cave, which were monitored at 15-minute intervals using acoustic drip loggers (Stalagmate<sup>®</sup> Plus Mk 2b, <u>http://www.driptych.com/</u>) measuring 55 mm × 55 mm × 65 mm. For each drip site, a Stalagmate was placed underneath the stalactite in a plastic funnel sitting in an HDPE bottle surrounded by a plastic tube to fix it in position. The minimum distance between the stalactite and Stalagmate was 25 cm. Site 03 slowly stopped dripping, while Sites 08–10 were misaligned during the dry periods.

## 2.3.6 Geochemical Analysis

Both the long-term and short-term drip water samples collected were first filtered through 0.45  $\mu$ m mixed-cellulose filters into 10 ml plastic sampling vials. Stable isotope samples were stored with zero-headspace. Two drops of ACS reagent HNO<sub>3</sub> acid (70%) were added into the cationic samples to prevent precipitation. All the prepared analytes were refrigerated at < 5 °C until analysis.

In-cave water samples were analysed to determine the  $\delta^2$ H and  $\delta^{18}$ O values, with the results given in per mille (‰) using the conventional delta notation relative to VSMOW

(Vienna Standard Mean Ocean Water). A Los Gatos Research (LGR) Water Vapour Isotope Analyzer was used in the UNSW IceLab for the analysis of the  $\delta^2$ H and  $\delta^{18}$ O levels in cave drip samples. The ICELAB was graded excellent prior to the period of sample analysis (Wassenaar et al., 2018). All the isotope analytes were filtered through 0.45  $\mu$ m mixed-cellulose filters again before injection into isotope analyser machine. The manufacturer-quoted instrument precision was 0.2‰ for  $\delta^{18}$ O, and the actual precision was < 0.16‰. The samples were calibrated against five internal standards  $(\delta^{18}O: -19.5\% \text{ VSMOW}, -16.2\% \text{ VSMOW}, -13.4\% \text{ VSMOW}, -8.0\%, -2.7\% \text{ VSMOW}),$ with VSMOW-2 run as a primary standard. Some 109 precipitation samples were analysed at the ANSTO Environmental Isotope Laboratory using a cavity ring-down spectroscopy method on a Picarro L2120-I Water Analyser (reported accuracy of  $\pm 1.0\%$  for  $\delta^2 H$  and  $\pm 0.2\%$  for  $\delta^{18} O$ ). The lab runs a minimum of two in-house standards calibrated against VSMOW/VSMOW2 and SLAP/SLAP2 with samples in each batch.  $\delta^2$ H data was provided for an additional 75 precipitation samples at the UNSW ICELAB.

Cation (Ca<sup>2+</sup>, Fe<sup>2+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, Si<sup>2+</sup>, Ba<sup>2+</sup> and Sr<sup>2+</sup>) concentrations were determined using inductively-coupled plasma optical emission spectroscopy (ICP-OES; Optima<sup>TM</sup> 7300DV, PerkinElmer, Shelton, USA) and inductively-coupled plasma mass spectrometry (ICP-MS; NexION 300D, PerkinElmer, Shelton, USA) at the UNSW Analytical Centre, except for water samples from the final collection (9<sup>th</sup> May 2017), which were analysed using inductively-coupled plasma-atomic emission spectroscopy (ICP-AES; ICAP7600, Thermo Fisher) at the Australian Nuclear Science and Technology Organisation (ANSTO). Anion (Cl<sup>-</sup>, l<sup>-</sup> and SO<sub>4</sub><sup>2-</sup>) concentrations were determined using an ion chromatograph (Dionex DX-600) with a self-regenerating suppressor at the ANSTO facility.

# 2.4 Results

# 2.4.1 Hydrology Results

Drip volumes are mainly determined by the curvature radius of the stalactite they fall from (Collister and Mattey, 2008). Given the long intervals of sample collection, which normally cover at least one full recharge event, the volume of each drip was assumed to be consistent throughout this study. The overall drip water recharge responses to precipitation are illustrated in Figure 2.2. It shows a change in the drip pattern after the fire that was independent of precipitation. The baseline signals during the drip logger recording intermission was 0 (e.g. no drips).

Overall, post-fire discharge events were characterised by higher mean and peak flows that were of shorter duration than pre-fire discharge events. Increased peak recharge was observed at Sites 02, 04, 05, 07, 09 and 11 (Appendix 1) two weeks post-fire. The skewed hydrological peaks were shorter for post-fire recharge events than pre-fire ones. Conversely, there were no notable changes in dripping pattern for Site 01, which was not directly under the burnt area (see Figure 2.1 b).

The duration, peak rates, average rates, total drips, skewness and kurtosis for all individual recharge events are presented in Figure 2.3, which uses box plots to compare pre- and post-fire data. Post-fire total drips were less variable but not significantly different to pre-fire total drips. Both peak and average recharge rates increased noticeably, and were associated with decreased duration. Detailed analyses of the event hydrographs are presented in Section 2.5.1.



Figure 2.2 Line plots of the official precipitation records in Wombeyan Caves (a.), compared with the overall recharges on individual sites (b.). Recharge records at individual sites are presented using the colour in agreement with other figures. The timing of the fire is indicated by the vertical red line.



Figure 2.3 Box plots of descriptive statistics for all individual recharge events at the burnt sites: duration (a), peak drip rates (b), mean drip rates (c), total drip amount (d), and skewness and kurtosis (e).

## 2.4.2 Isotope Results

Isotopic data for the 184 precipitation events collected at Mt Werong from August 2014 to May 2017 (January 2015 was missing due to insufficient rainfall) show that the values for the  $\delta^{18}$ O samples (n = 109) ranged from -17.7% VSMOW to 1.3% VSMOW, and from -126.4% VSMOW to 34.1% VSMOW for the  $\delta^{2}$ H samples (n = 184; Figure 2.4). The weighted mean precipitation composition was isotopically heavier in the 12 months pre-fire ( $\delta^{18}$ O = -6.3% VSMOW,  $\delta^{2}$ H = -32.3% VSMOW) than the 12 months post-fire ( $\delta^{18}$ O =  $\sim -8.2\%$ ,  $\delta^{2}$ H = -48.2%).

A notable departure from this pattern occurred between Jun-August 2015 and May-Jun 2017, where the  $\delta^{18}$ O value was significantly lower than the long-term mean (see Figure 2.5). Post-fire, in June 2016, the lowest isotope values were acquired, and a prolonged period of rain occurred at both Mt Werong and Wombeyan Caves, where 238 mm fell over 22 raindays, which is more than three times the long-term mean precipitation for June (69.9 mm). This was associated with two consecutive east coast low-pressure systems affecting the NSW coast in one month, resulting in record high daily and monthly rainfall records at many stations. The rain from these two systems was significantly isotopically lighter, with a monthly  $\delta^{18}$ O mean of –14.5‰ VSMOW and a  $\delta^{2}$ H mean of –98.7‰ VSMOW in June 2016.



Figure 2.4 Time series of monthly weighted mean rainfall at Wombeyan, Mt Werong and tipping bucket rain gauge (TBRG) records in Mt Werong, mean rainfall isotopes from Mt Werong stations. D-excess on rainfall is also displayed. The black circles represent estimates of  $\delta^{18}$ O for months where records were incomplete. The vertical yellow line indicates the timing of the experimental fire (25<sup>th</sup> May 2016).

Drip water  $\delta^{18}$ O values for 213 samples are presented in Figure 2.4 which reflect the residual time in the vadose zone and changes in precipitation (Cuthbert et al., 2014; Jouzel et al., 2000). Until May 2016,  $\delta^{18}$ O values ranged from -8.5‰ VSMOW to -3.1‰ VSMOW, and  $\delta^2$ H values ranged from -50.6‰ VSMOW to -10.1‰ VSMOW. After the fire, sites behaved differently according to their locations. For the shallower sites (Sites 07–11), there was a response the day after the burn, with the mean  $\delta^{18}$ O composition being 4.5‰ lower than that for all pre-fire samples (which ranged from -5.1‰ VSMOW to -9.6‰ VSMOW). In contrast, there was only a slight difference in  $\delta^{18}$ O at Sites 02–06

(-5.5‰ VSMOW to -6.2‰ VSMOW). Two weeks later, following 115.6 mm of precipitation from 27 May to 3 June 2016, the  $\delta^2$ H isotope in Sites 07–11 increased by 0.6 ‰, while at Sites 02–06 it increased by 0.7‰ on average. In terms of O isotopes, both groups had decreased  $\delta^{18}$ O, by 0.8‰ and 0.6‰, respectively.



Figure 2.5 Time series of the  $\delta^2$ H (a.) and  $\delta^{18}$ O (b.) of drip water. The experimental fire is indicated by the red line). The rainfall data was presented in figure 2.4. Mean isotope values for local groundwater ( $\delta^2$ H = -38.45‰ VSMOW ±5.10‰,  $\delta^{18}$ O = -7.31‰ VSMOW ± 0.59‰) are presented as the horizontal blue line.

The largest shift occurred during the next sampling campaigns (5 and 21 June 2016). All of the ten burnt sites were significantly isotopically lighter (-76.5‰ and -84.1‰ in  $\delta^2$ H for Sites 02–06 and 07–11, respectively, and -11.9‰ and -12.7‰ in  $\delta^{18}$ O). One month later, the sites showed a recovering trend until a new peak was reached in March 2017. The control site (Site 01) was consistently lighter than its pre-fire mean values. After December 2016, drips at the control site were isotopically lighter than those at the

burnt sites. Over the post-fire hydrological year, drip water became more isotopically depleted than before the fire. In addition, the lower d-excess verified that the fire depleted the stores, such that old water which would have been affected by evaporation was removed and the stores were replenished with new recharge.

Three groundwater samples from the borehole in Wombeyan camping area were collected, on 23 September 2015, 11 Jan and 23 March 2017, and had an average  $\delta^2 H$  of -38.45 ± 5.10 and an average  $\delta^{18}$ O value of -7.31 ± 0.59 (Figure 2.5, shaded blue). This shows there was little change in groundwater values over the last 2.5 years.

The pre-fire drip water stable water isotope composition was above the global meteoric water line (GMWL; Figure 2.6 The effect of fire on the combined  $\delta^{2H}$  and  $\delta^{180}$  data for pre-fire (a.) and post-fire samples (b.).). The local meteoric water line (LMWL) was established using precipitation data from Mt Werong. Three different groups based on their spatial locations in the cave were plotted separately in Figure 2.6.a and Figure 2.6.b. In Figure 2.6.a, the slope for Sites 07–11 (slope = 6.5) was similar to the LMWL (slope = 6.6), while the slope of Site 01 slightly lower (slope = 5.8). Moreover, the borehole groundwater showed a 1.0‰ decrease in  $\delta^{18}$ O from Sep 2015 to Jan 2017. As a direct consequence, the slope of unburnt Site 01 increased from 5.8 pre-fire to 7.4 post-fire.



Figure 2.6 The effect of fire on the combined  $\delta^2 H$  and  $\delta^{18} O$  data for pre-fire (a.) and post-fire samples (b.).

# 2.4.3 Elemental Results

Sixty-two opportunistic samples were collected when the cave was dripping during the bi-monthly sampling campaigns. There were fewer samples collected in the dry cold months, especially after fire. In the post-fire groups, we succeeded in acquiring ten drip samples the day after the fire, then six more in the first month (see Table 2.1, '1

mth post-fire'). After that, water samples were collected in Dec 2016, and Jan and Mar 2017 (see Table 2.1, '6 mth post-fire').

Results of Mann-Whitney U tests showed there was a significant difference between different time periods for some drip water elemental concentrations (see Table 2.1 Descriptive statistics and Mann-Whitney U test results for elemental concentrations. Samples are separated into three groups (pre-fire, 1 month post-fire and 6 months post-fire). Statistical significance (p < 0.05) is indicated IN BOLD TEXT). The concentrations of all bedrock-derived elements in drip water (Ba<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and Sr<sup>2+</sup>) were significantly lower after the fire. Barium did not change immediately but decreased after six months, whereas Ca<sup>2+</sup>, Mg<sup>2+</sup> and Sr<sup>2+</sup> were lower one month and six months post-fire. The decreases in concentrations of Ca<sup>2+</sup>, Mg<sup>2+</sup> and Sr<sup>2+</sup> were substantial one month post-fire and appeared to recover six months post-fire. Cl<sup>-</sup> remained lower after the fire. SO4<sup>2-</sup> increased 10-fold one month post-fire and although it decreased between one and six months post-fire, it remained above prefire levels. Iodide slightly increased initially, but returned to below the pre-fire level half a year later.

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Table 2.1 Descriptive statistics and Mann-Whitney U test results for elemental concentrations. Samples are separated into three groups (pre-fire, 1 month post-fire and 6 months post-fire). Statistical significance (p < 0.05) is indicated in bold text.

lon	Sampling	N	Min	Q1	Median	Q3	Max	U	Ζ	Exact Proballul
	Pre-fire	12	1.4	3.9	4.4	6.4	23.3	-		1100/101
Ba /mg·L <sup>-</sup> 1	1 mth post- fire	13	1.6	2.3	3.0	12.8	30.1	105	1.4	0.2
	6 mth post- fire	10	2.0	2.0	2.0	4.3	6.0	94	2.2	0.0
Ca /mg·L <sup>-</sup> 1	Pre-fire	40	47.0	87.2	107.9	119.1	150.5	-		
	1 mth post- fire	14	49.4	57.1	65.8	74.4	81.9	525	4.8	0.0
	6 mth post- fire	10	67.9	75.4	91.8	99.8	110.0	288	2.1	0.0
	Pre-fire	29	0.4	0.8	1.3	1.5	2.3	-		
Mg ∕mg∙L⁻ ¹	1 mth post- fire	14	0.6	0.7	0.7	0.8	1.4	311	2.8	0.0
	6 mth post- fire	10	0.7	0.8	1.1	1.2	1.6	179	1.1	0.3
Sr / g·L <sup>-</sup>	Pre-fire	38	1.4	61.4	72.5	84.5	109.4	-		
	1 mth post- fire	13	0.2	36.2	40.1	71.7	78.9	384	3.0	0.0
	6 mth post- fire	10	37.0	46.8	50.5	57.8	64.0	10	3.5	0.0
	Pre-fire	20	1.1	1.6	1.8	2.0	2.6	-		
Na ∕mg∙L⁻ ¹	1 mth post- fire	14	1.0	1.2	1.4	2.0	2.0	190	1.7	0.1
	6 mth post- fire	10	1.1	1.2	1.3	1.9	2.5	142	1.8	0.1
Cl /mg·L <sup>-</sup> 1	Pre-fire	27	1.2	2.5	3.1	4.0	11.6	-		
	1 mth post- fire	14	2.1	2.7	4.5	6.7	13.1	12	- 1.5	0.1
	6 mth post- fire	10	2.7	2.9	3.7	6.5	14.0	101	- 1.2	0.3
K /mg·L <sup>-</sup> 1	Pre-fire	21	0.1	0.6	0.8	3.0	25.2	-		
	1 mth post- fire	14	0.1	0.1	0.4	1.0	1.5	218	2.4	0.0
	6 mth post- fire	10	0.1	0.1	0.2	0.3	1.2	190	3.6	0.0
Si	Pre-fire	39	1.0	1.5	2.0	2.0	2.3	-		
/mg·L⁻	1 mth post-	14	1.0	1.1	1.2	1.3	1.4	516	4.9	0.0

1	fire									
	6 mth post- fire	10	1.2	1.4	1.5	1.6	1.8	303	2.7	0.0
I ∕mg∙L⁻ ¹	Pre-fire	19	0.1	0.1	0.3	1.5	7.3	_		
	1 mth post- fire	8	0.1	0.5	1.0	1.3	1.5	61	0.8	0.4
	6 mth post- fire	10	0.1	0.1	0.1	0.1	0.1	180	4.0	0.0
	Pre-fire	27	0.0	0.1	0.1	0.2	0.4	-		
SO4 /mg·L <sup>-</sup> 1	1 mth post- fire	14	0.3	0.4	1.2	6.2	20.6	3	- 5.1	0.0
	6 mth post- fire	10	0.4	0.6	0.8	1.4	2.6	0	- 4.6	0.0
Fe /mg·L <sup>-</sup> 1	Pre-fire	35	0.6	1.5	2.0	2.8	5.5	-		
	1 mth post- fire	14	0.9	1.1	2.0	3.2	9.7	243	0.0	1.0
	6 mth post- fire	6	0.0	0.0	0.0	0.0	0.0	210	3.9	0.0

#### 2.5 Discussion

### 2.5.1 Cave Drip Hydrology and Karst Architecture

Wombeyan Limestone is affected by post-depositional metamorphism, which causes the marmorisation of the limestone (Brunker and Offenberg, 1970). Overall, there is no sign of any original structure in the marble and there is little primary porosity. Preferential flow in the soil and overflow or bypass flow in the karst dominates cave recharge in Wildman's Cave. Theoretically, discharge from soil storage would be buffered by soil capillarity (Fredlund and Rahardjo, 1993) resulting in moderate drip rates and diffuse or capillary flows to the karst and cave below. When there is no interaction between the soil water store and the bedrock, discharge only occurs from the karst water store and drip rate peaks are likely to decline more rapidly.

The post-fire recharge in Wildman's Cave was more intense, having a higher magnitude, shorter duration and less frequent hydrographic peaks than the pre-fire recharge. By comparing log10-transformed changes in drip rates over time, the drainage stages during the recession stage of individual recharge events can be identified. These stages are represented in Figure 2.6, and all drip data are presented in SUPPLEMENTARY FIGURE 1.2. DRIP RECORDS FROM INDIVIDUAL DRIPPING SITES. Through the changes of the slopes (the log10-transformed rates of decrease in recharge), we can interpret the changes to the dominant recharge flow patterns at each site. Three different stages in the recession stage of recharge for individual precipitation events are identified.

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- a) In the first stage (Figure 2.7, green), when both the soil and karst are saturated with event water, soil moisture is above field capacity, which permits the maximum possible recharge rate from the soil to the karst stores. The major pathway for the event water is through preferential flows in the surface soil directly to the karst fractures, with bypass or overflow of the karst stores. Flow rates are mainly restricted by the minimum diameter of the fracture in the karst, or the internal diameter of the stalactite.
- b) In the second stage (Figure 2.7, blue), the soil is no longer saturated enough to support preferential flow in the soil and overflow or bypass flow in the karst. Capillary flow from the soil into the karst stores becomes the dominant flow pathway. In this way, relatively slower recharge rates from the soil, and longer residence time in karst stores, generate shallower slopes. In some cases, especially in the inner cave regions like Site 07, this stage is less frequently observed or even mixed with the first stage. We hypothesise that this is due to lower soil water storage volumes and weaker capillarity effects from a thinner soil layer.
- c) In the final stage (Figure 2.7, yellow), when the surface precipitation has stopped and soil moisture falls below field capacity, drainage to the karst by capillary flow also stops. The amount of new event water is now low. The cave drips are quickly recharged from the residual karst stores, and the drip rate is again limited by the diameters of the fractures or stalactites.

Fires can change the physical and chemical properties of soil (Bonacci et al., 2008). An increase in soil hydrophobicity can affect flow paths within the soil (Huffman et al.,

2001). Stoof et al. (2014) reported that fire-induced preferential flows are associated with a change in soil water repellency. The total storage volume is unchanged post-fire, with similar total recharge amounts pre- and post-fire (see Figure 2.3 c). However, a post-fire increase in preferential flow in the soil could explain the observed change in drip rates in the three stages described in Figure 2.6.



Figure 2.7 Conceptual models for the fire influence on vadose zone discharge events during individual precipitation events. Y-axis is displayed in log10 transformation. Stages a, b, c are shaded in green, blue, yellow, respectively.

Three effects of fire on the recession stages (Figure 2.2.7) are described below:

Effect 1) For the deeper sites, which were covered with a thicker soil layer (Sites 02-

04), increases in mean post-fire drip rates were found for all precipitation

events across all the stages of recession. The peak flow rates increased

slightly. Fire-induced increases in soil preferential flow pathways increased the drip rates in the first stage. In the second stage, an increase in soil hydrophobicity weakened the soil's capillary action, which substantially decreased the duration of the capillary flow controlled stage. In the final recession stage, the karst stores were unaffected by the surface fire, and there was no change in the draining of the last water from the fractures.

- Effect 2) For the shallower sites (Site 07), the effects of changes in soil hydrophobicity and preferential flows were amplified. At Site 07, soil capillary flow was the dominant process pre-fire, and the preferential flows were not obvious. Post-fire, newly created preferential flows dominated the capillary flows, so that the drip rate for this site was mainly controlled by the first stage (a).
- Effect 3) For sites in the middle part of the cave, deeper than Site 02 and with a thinner soil layer than Site 01 (e.g. Site 05), there was a similar pre-fire drip rate response to that at Sites 02–04. Increased preferential flows dominated the capillary flows which originally appeared in stage (b).

Both Sites 06 and 11 were fed by two asynchronously dripping stalactites, making it difficult to identify the specific stages. However, increased preferential flows possibly still occurred at both sites. We propose that the fire-induced increase in preferential flows and the loss of soil capillarity both led to more intense cave drip recharge.

## 2.5.2 Stable Isotope Behaviour and Residence Time of Drip Waters

The overall mean drip water  $\delta^{18}$ O results are compared with the precipitation at Mt Werong for each recharge period in Table 2.2, with the precipitation amount

compared to each drip water collection period. Wildman's Cave drip water  $\delta^2 H$  and  $\delta^{18}$ O values were less variable than Mt Werong precipitation values. The Mt Werong precipitation was isotopically lighter during the post-fire period. This relatively isotopically light precipitation contributed to the post-fire isotopic composition of the percolation water that flowed into the cave. The figures in Section 2.4.2 show that most of the high rainfall months before and after the fire (e.g. Dec 14, Jan 15, Apr 15, Jan 16, Mar 17) had isotope compositions quite similar to the drip compositions existing at those times. Where rainfall amounts departed from this, there was a trend in drips over a few months. Where rainfall amounts were small, the response was slow, e.g. from May to October, 2015 drip waters become gradually lighter and from October 2016 to March 2017, they become heavier. Even following the isotopically heavier and higher rainfall months of December 2014 to January 2015, there was no apparent response in drip water composition until in March 2015. This also reflects a lagged or gradual response to the usual seasonality.

Pre-fire, the mean  $\delta^{18}$ O values of Wildman's Cave drips showed similar changes over time to those of precipitation. Post-fire, the mean  $\delta^{18}$ O values of drips lay within the mean ± standard deviation (SD)  $\delta^{18}$ O precipitation values. The decrease in stable isotope ratios could be attributed to (a) changes in the precipitation  $\delta^{18}$ O, (b) changes in the soil's physical properties, (c) decreased transpiration due to vegetation loss and (d) the evaporation of old water within the soil and karst stores.

Start Time	Date to Collect	Rainfall at Wombeyan (mm)	Mean δ <sup>18</sup> O of Wildman's Cave (‰ VSMOW)	SD (‰)	Rainfall at Mt Werong (mm)	Mean δ <sup>18</sup> O of Mt Werong (‰ VSMOW)	SD (‰)
22/05/2015	29/06/2015	58	-4.8	0.4	3	-5.1	N/A
29/06/2015	23/07/2015	59.4	-5.1	0.8	62.5	-12.0	3.1
23/07/2015	24/08/2015	23.8	-5.8	1.2	128	-6.4	4.0
24/08/2015	23/09/2015	108	-6.7	0.5	28	-4.1	2.3
23/09/2015	23/11/2015	119	-6.2	0.8	175	-2.0	2.3
23/11/2015	27/01/2016	142	-4.6	1.0	209	-4.5	3.2
27/01/2016	22/03/2016	100.4	-4.2	0.6	55	-4.2	1.5
22/03/2016	26/05/2016	54.4	-3.5	0.5	73.5	-5.8	2.0
27/05/2016	5/06/2016	118	-8.0	2.5	119	-11.2	5.6
5/06/2016	21/06/2016	97.2	-11.8	1.7	85	-10.0	2.7
21/06/2016	17/08/2016	139	-9.1	0.5	161	-9.2	4.1
17/08/2016	9/11/2016	205.8	-8.7	1.0	170	-7.3	2.6

Table 2.2 Mean  $\delta^{18}$ O concentration of drips in Wildman's cave and of rainfall at Mt Werong over different sampling periods.

A large precipitation event (164.0 mm), which occurred one week post-fire, could have contributed to a considerable amount of the isotopically light recharge to the cave. As a result, the calculated mean  $\delta^{18}$ O concentration of cave drips within this period was = -8.0 ‰ VSMOW ± 2.5 ‰, compared to the mean  $\delta^{18}$ O concentration of precipitation of -11.2 ‰ VSMOW ± 5.6 ‰. While slight changes in the correlations between  $\delta^{2}$ H and  $\delta^{18}$ O occurred within that period, the  $\delta^{2}$ H concentration of all burnt sites increased by 0.6 ‰ while that of  $\delta^{18}$ O decreased by 0.7 ‰. However, the variation in precipitation  $\delta^{18}$ O can be partly ruled out as the major contributing factor, because the drip water  $\delta^{18}$ O values in June were lighter than those of precipitation ( $\delta^{18}O_{drip}$  = -11.8 ‰ VSMOW ± 1.7 ‰;  $\delta^{18}O_{precipitation}$  = -10.0 ‰ VSMOW ± 2.7 ‰). This corresponds the

interpretation provided in Section 5.1: that increased preferential flow post-fire also decreased the cave recharge duration. Consequently, drip rates increased and most of the water took less time to travel to the cave. Thus, less evaporation could occur along the flow paths, which largely preserved the precipitation isotope composition during post-fire cave recharge events.

A change in drip water  $\delta^{18}$ O and  $\delta^{2}$ H could occur if the evaporative conditions in the soil, prior to infiltration, were changed by the fire. The data suggest that larger and more intense precipitation events occurred after fire and that precipitation  $\delta^{18}$ O amplified the large isotopic shift (Figure 2.4). As interpreted above, the initial post-fire changes were the complete evaporation of residual soil waters, which were quickly reflected at Sites 07–11 and less so at Sites 02–06. The complete evaporation of soil water should be the main driving force of the large isotopic shifts. This influence was related to the depth of the dripping sites. Sites 02–06 were deeper than Sites 07–11, and the loss of preserved old soil water in the fire event was buffered by the deeper karst water stores with immediate -0.7‰  $\delta^{18}$ O changes at Sites 02–06 compared to -4.5‰  $\delta^{18}$ O changes at Sites 07–11. Moreover, Figure 2.5 shows that the largest preferential flow changes occurred among Sites 07-11, changing the slope of its meteoric water line from 6.5 pre-fire to 7.9 post-fire (see Figure 2.5). As a comparison, the slope of Sites 02–06 slightly increased from 7.2 to 7.3. Thus, we confirm that, besides the complete removal of soil water, an increase in preferential flows and a decrease in shallow karst stored water were the major influences of the fire on the shallow cavern system.

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The loss of vegetation can cause a positive isotopic shift in cave drip water (Nagra et al., 2016). Burnt areas with less vegetation have a lower albedo, which can increase soil water evaporation. This causes enrichment with respect to <sup>18</sup>O/<sup>17</sup>O in soil water, which can then discharge to the cave. This factor potentially boosted the recovery trend based on water isotopes. After six months, drip water  $\delta^{18}$ O showed that the sites underneath the burnt areas had more negative  $\delta^{18}$ O values than Site 01, which was outside of the burn area. With time, surface soil and nutrient biomass re-accumulated and the temporary loss of old water recovered.

# 2.5.3 Trace Elemental Signatures

Unlike the isotope signals that showed a consistent post-fire response consisting of an initial rapid change then a recovery trend, the solutes showed much greater variability between sites. The hydrological interpretation of Section 5.1 indicates a decrease in water residence time, which is associated with decreased dissolution of bedrock and subsequent precipitation of calcite (Fairchild and Treble, 2009). Bedrock-related elements including Ca<sup>2+</sup>, Mg<sup>2+</sup> and Sr<sup>2+</sup> decreased one month post-fire with *Z*-scores of 4.83, 2.79 and 2.95, respectively, in Mann-Whitney *U* tests with a 95% confidence level (see Table 2.2). This could reflect a decrease in carbonate dissolution due to the evaporation of old storage water and a shorter water residence time. Decreased soil CO<sub>2</sub> from the destruction of plant roots and microbes could also enhance this effect.

Comparison of Mg/Ca and Sr/Ca ratios can be used as a geochemical signature of bedrock dissolution. These did not show any notable changes within the study timescale for the pre- and post-fire groups (see Figure 2.8 Time series of ratios of Sr/Ca

(a.), Mg/Ca (b.), and In(Sr/Ca) vs. In(Mg/Ca) (c.)). It suggests that, for such a shallow cave where the new inputs could barely saturate the calcite dissolution, each precipitation event varied in residence time and recharge volume, failing to provide a constant correlation with the surface conditions.



Figure 2.8 Time series of ratios of Sr/Ca (a.), Mg/Ca (b.), and  $\ln(Sr/Ca)$  vs.  $\ln(Mg/Ca)$  (c.)

Despite ash being present across the fire site, there was only limited geochemical evidence of ash-derived elements in the drip waters. Sulfate drip water concentration significantly increased after fire, whereas concentrations of other analysed cations decreased after the fire experiment. The lack of ash-derived elements in the drip water could be due to the volatilisation of elements at high temperatures (Bodí et al., 2014). The fire temperature exceeded 929 °C in some areas (see Appendix 1) which could volatilise organic compounds leaving white ash, which was observed at the experiment site (Figure 2.1). Moreover, 1<sup>-</sup> and Cl<sup>-</sup> concentrations both increased slightly with *Z*-scores of -0.77 and -1.53, respectively, and 1<sup>-</sup> concentration changed significantly half a year post-fire. Concentrations of Cl<sup>-</sup> also indicated the removal of soil water by the fire. In summary, the combustion of vegetation and soil fauna is hypothesised to lead to a decrease in limestone dissolution (lower Ca<sup>2+</sup>, Sr<sup>2+</sup> and Ba<sup>2+</sup>) and a decrease in soil- and vegetation-derived elements. Nutrition elements from ashes were largely volatilised.

### 2.6 Conclusion

This research has demonstrated the impacts of a high-severity experimental fire on the karst vadose zone, including 1) short-term complete evaporation of soil water; 2) increased preferential flows and decreased capillary flows; and 3) increased soil hydrophobicity. Three different stages of discharge were defined based on the hydrograph analysis on the cave drip rates in this study. Based on that, more intense stage 1 post fire was attributed to the increased preferential flows, and decreased stage 2 to the decreased capillary flows.

The short-term responses observed in cave drip water were large negative shifts in stable water isotope values and decreased concentrations of bedrock-derived elements within the first month post-fire. Lighter isotopes show that old soil water was evaporated by the heat, while a shorter water residence time resulted in less enriched signals with respect to  $\delta^2$ H and  $\delta^{18}$ O. A recovery trend, which was potentially accelerated by the decreased albedo, was observed through the consistently more negative water isotopes within the first six months post-fire. After that, isotope compositions were slightly heavier.

The bedrock-related element concentrations decreased after the fire experiment because of lower recharge durations and potentially decreased partial pressure of carbon dioxide (pCO<sub>2</sub>). Ash products were largely volatilised due to the severe intensity of the fire and were not captured by the cave, instead leaving white-coloured ash above the cave.

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# CHAPTER 3 Organic Carbon Isotope Analysis: An Innovative Method and Application to Speleothem

# Abstract

Organic matter in speleothems has the potential to reveal changes in the environment above the cave. In the past,  $\delta^{13}$ C analysis applied to speleothems could only measure the total  $\delta^{13}$ C in carbonate as a combination of organic sources (compounds derived from soil and cave organisms) and inorganic sources (calcite from dissolved bedrock present as dissolved HCO<sub>3</sub>). In this work, an innovative  $\delta^{13}$ C analysis method was proposed for detecting organic matter entrapped in speleothems. It was applied to Easter Cave Flowstone 1 to investigate the function of organic  $\delta^{13}$ C values in paleoclimate records. Speleothem powder samples (30 mg) were dissolved in 1 ml of 3 M phosphoric acid to produce an aqueous solution. After half an hour degassing, the processed solution was oxidised and measured via liquid chromatography isotope ratio mass spectrometry. Our results demonstrate that this proposed method is reliable for the analysis of speleothems under constant preparation and instrumental conditions, as there was a relatively low average standard deviation (~0.2%) and low sample consumption (~25 mg). The organic  $\delta^{13}$ C values remained constant over its growing period, indicating a consistent ratio of C3 and C4 plants during almost half of the Holocene period. Fractionation is the main influence on calcite  $\delta^{13}$ C variation.

#### 3.1 Introduction

Speleothems can be formed from incremental laminations, which can provide a record of annual climate changes over short or long time-scales. In the short term, manual or automated counting of growing laminations can contain information at sub-annual resolutions (Baker et al., 2008). At the other end of the scale, they are highly amenable to chemical dating, potentially providing a record of several million years (Hellstrom, 2006; Hellstrom and Pickering, 2015).

Precise dates can be detected through uranium series analysis, and speleothems contain geochemical signatures, including carbon and oxygen isotopes, trace elements and organic matter. Analysis of calcite  $\delta^{18}$ O is now recognised to be potentially ambiguous due to a number of controlling variables (McDermott, 2004). In clumped isotope analyses, it was found that there is disequilibrium in calcite precipitation proceeded by kinetic fractionation, leading to underestimates of temperature (Daëron et al., 2011).

Speleothems contain two different pools of carbon sources: carbonate and entrapped organic matter. Organic matter (OM) in speleothems are derived from in-cave microbial activity and surface flora, with potential to reveal changes in the environments above and within caves. The study of OM in speleothems has been conducted for more than two decades in order to expand the range of chemical proxy records recovered, including those deriving directly from plant and microbial communities. The amount of organic carbon preserved in speleothems is low, estimated at 0.01–0.3% of total carbon (Blyth et al., 2013; Li et al., 2014; Quiers et al.,

2014). To incorporate organic matter into paleoclimate records, firstly, the meaning of calcite  $\delta^{13}$ C needs to be understood. Changes of  $\delta^{13}$ C in calcite are difficult to interpret without another proxy like the dead carbon proportion (DCP).  $\delta^{13}$ C can change according to the relative proportion of C3 vs. C4 plants, conditions of limestone dissolution, or changes in soil CO<sub>2</sub>  $\delta^{13}$ C composition due to variations in mixing with atmospheric CO<sub>2</sub> (Blyth et al., 2016). Figure 3.1 displays the chemical evolution of soil  $CO_2$  from  $C_3$  vegetation [ $\delta^{13}C = -23\%$  Vienna Pee Dee Belemnite (VPDB)] and from  $C_4$  $(\delta^{13}C = -9\% \text{ VPDB})$  vegetation providing high  $(10^{-1.5})$  and low  $(10^{-2.5})$  soil pCO<sub>2</sub> conditions at 25 °C. The results were calculated using the data in Drever (1997). Roughly, the enrichment reflects a 50:50 mixture between the  $\delta^{13}$ C of the dissolved CO<sub>2</sub> and the calcite for closed system evolution. As interpreted, an equilibrium system with  $\delta^{13}\text{C}$  fully provided by C3 plants can supply organic  $\delta^{13}\text{C}$  ranging from -12.1‰ VPDB to -16.8‰ VPDB. C4 plants dominated system could provide organic  $\delta^{13}$ C from -16.8‰ VPDB to -2.9‰ VPDB. Thus, organic  $\delta^{13}$ C values could be powerful evidences to understand the function of surface vegetation in the paleoenvironmental reconstruction.



Figure 3.1 Evolution of dissolved inorganic carbon (DIC) and  $\delta^{13}C_{dic}$  in groundwater as calcite ( $\delta^{13}C = 0$ % VPDB) is dissolved to the point of saturation. After Fritz and Clark (1997).

Bulk OM analysis has rarely been attempted, partially due to the difficulty of separating it from the acid digest without retaining acid salts, losing the water-soluble portion of the sample, fractionating the signal or adding carbon contamination. Moreover, potential damage from residual acid salts can damage instruments such as elemental analysers. Although there are adequate investigations into the methodology of  $\delta^{13}$ C analysis in carbonate, a simple and well-developed micro-analytical method for detecting the organic matter in stalagmites remains lacking. Blyth et al. (2013) conducted a preliminary study using liquid chromatography isotope ratio mass spectrometry (LCIRMS) to analyse stalagmite powder. In 2014, Li et al. (2014) performed a 9000-year acid-soluble organic matter isotope study by analysing 2 g dissolved stalagmite samples with an elemental analyser (EA; Thermo Flash) and an isotope ratio mass spectrometer (IRMS; Thermo MAT-253).

This study comprised two parts: 1) design and optimisation of methodology as an extension to Blyth et al. (2013); and 2) application of the method to comparison of calcite isotopes and organic matter series studied in parallel on Easter Cave Flowstone 1, over the time-scale 20 ka to modern times. Influences on fluctuations in the calcite  $\delta^{13}$ C series are separately discussed in relation to the surface environment, climate and fractionation factors over time.

#### 3.2 Method Development and Site Description

#### 3.2.1 Flowstone Sampling

The flowstone powders used in the paleoenvironment study were acquired from Easter Cave, Augusta, WA. The Augusta karst area comprises the southern-most section of the Leeuwin-Naturaliste Ridge between Turner Brook and Cape Leeuwin, and is near the township of Augusta. This section of the limestone ridge is up to 3.5 km wide and 14 km in length, with a surface area of about 40 km and an elevation of up to 210 m above sea level.

Easter Cave is part of the large Jewel-Easter sub-system containing Easter Cave, Jewel Cave, Moondyne Cave, Skull Cave, and eight other caves. Specifically, it is one of the Augusta water table caves and occurs within a 5 km-wide belt known as the 'cave belt' with a vertical pothole type entrance.

ESF1 (Easter Cave Flowstone 1) had been previously sampled near the 'Y-junction' in Easter Cave (intersection of the Epstein passage and the Main Drag). Heavy minerals were evident on the wall of the cave passage near the Y-junction. The ceiling of the passage is a palaeosol or fissure fill consisting of limestone clasts and bones (not visible) within a well consolidated 'terra rossa' matrix.

# 3.2.2 Preparation and Analytical Method

For method development, a homogeneous stalagmite powder used. This was from a strongly-laminated specimen collected from Lower Traligill Cave in Assynt, northwest

Scotland in 2003, as the extension of a 2000-year lipid biomarker study and a preliminary study of organic  $\delta^{13}$ C (Blyth et al., 2011, 2013). Certain amounts of homogeneous stalagmite powder were dissolved by phosphoric acid at different concentrations to produce aqueous analytes. After 30 mins of thorough degassing through a rotational vacuum concentrator, the treated substances were incorporated with a mobile phase, a catalyst and an oxidant. The process was controlled by an LC-Isolink and an Accela autosampler and final signals were acquired by an isotope ratio mass spectrometer (Delta V plus).

Different conditions were applied to test the replication of carbon isotope signals under different analytical atmospheres. The raw method was proposed by Blyth et al. (2013), and then adapted in the study by Li et al. (2014); the reference standard used was USGS-40 with  $\delta^{13}$ C of -26.39‰ VPDB. Liquid chromatography flow rates for the catalyst (1.28 mol/L H<sub>3</sub>PO<sub>4</sub>) and oxidant (0.13 mol/L Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) were both initially set to 50 µl/min. Dilute H<sub>2</sub>SO<sub>4</sub> of pH 4.0–4.2 (made by adding 100 ml of 1:50 H<sub>2</sub>SO<sub>4</sub> to 1 L of MilliQ water) and a 300 µl/min flow rate was employed as the mobile phase, and 100 mg calcite power was dissolved in 1 ml 3 mol/L (M) phosphoric acid for use as an analyte after degassing under a vacuum for 0.5 hr.

A different mobile phase (0.3%  $H_3PO_4$ ) was also tested. At the second stage, injection volumes were decreased to 5 µl in order to use the best analytical ranges of the IRMS machine (1–8 k signal amplitudes). Three levels of acid concentration were prepared to optimise the preparation conditions at this stage: 1 M, 2 M and 3 M phosphoric acid. As the last stage, the amount of the initial stalagmite powder was decreased to 25 mg

to extend its application ranges. A detailed table of the experimental conditions can be found in SUPPLEMENTARY TABLE 2.1. CONDITIONS AND RESULTS OF METHOD DEVELOPMENT USING THE USGS-40 STANDARD..

As a first attempt, four random samples (with depths above the bottom of the ESF1 = 180 mm, 179 mm, 172 mm, 165 mm) were selected to test the method's reliability, with flow rates of catalyst (1.28 mol/L H<sub>3</sub>PO<sub>4</sub>) and oxidant (0.13 mol/L Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub> solution) set to 30  $\mu$ l/min. Dilute H<sub>2</sub>SO<sub>4</sub> (pH 4.0–4.2) with a 300  $\mu$ l/min flow rate was employed as a mobile phase. Hydrate acid was 1 ml 3 M phosphoric acid. Injection volumes were set to 5  $\mu$ l.

# 3.2.3 Analysis of Calcite Isotopes

The ages for the ESF1 series were provided for this project, dated using Nu Plasma MC-ICP-MS in University of Melbourne following the measurement proposed by Hellstrom (2003). The dual-inlet mass spectrometer in Bioanalytical Mass Spectrometry Facility, UNSW was used for the calcite  $\delta^{18}$ O and  $\delta^{13}$ C analyses, with data also provided for this project. 20–30 µg of samples were weighed out and analyzed on a MAT 253 isotope ratio mass spectrometer equipped with a Kiel IV carbonate device (Thermo Fisher Scientific, Bremen, Ger). Standards NBS18 and NBS19 (IAEA) were used for data correction on each day of analysis. The full method was also introduced in Ji et al. (2016).

### 3.3 Results

## 3.3.1 Method Optimisation

First, the optimism of analytical conditions was tested. Figure 3.2 displays the scatter plots of results with different acquired amplitudes. When the results provided amplitudes higher than 11 V with both the injection and preparation using the max volumes, the results were more likely to be affected by different reaction conditions (SD of raw  $\delta^{13}$ C = 0.33‰ VPDB, SD of corrected  $\delta^{13}$ C = 0.40‰ VPDB). One possible explanation is that the variations in the instruments were amplified with an amplitude of the mass spectrometer higher than the instrument's allowance. The amplitude acquired with 5 µl injection volume provided lower SDs, from ±1103 mV to ±192 mV on standard samples.

Also, the SDs of  $\delta^{13}$ C decreased from 0.40‰ VPDB to 0.24‰ VPDB, with heavier isotope signals from -22.87‰ VPDB to -21.98‰ VPDB. When the least amount of samples were injected (5 µl injection with 25 mg powder), this method was proved to be the most reliable regardless of which parameter was changed ( $\delta^{13}$ C = -22.0‰ VPDB ± 0.27‰).



Figure 3.2 Scatter plots showing the changes in raw  $\delta^{13}$ C concentration of homogenous stalagmite powder (a.),  $\delta^{13}$ C with offsets corrected by reference USGS-40 (b.), and amplitudes of reference USGS-40 (c.) responding to different experimental settings.
### 3.3.2 Application to Easter Cave Flowstone

#### 3.3.2.1 Organic Matter Concentrations

Three different groups were analysed: 0–10 mm, 89–97 mm and 137–146 mm (depths above the bottom of the ESF1). For the IRMS, acquired amplitudes varied according to the mass of the preparation, the volume of the injection and the concentrations of organic matter in the flowstone. Thirty milled powder samples from ESF1 were analysed using the method proposed in Section 3.2 to acquire their organic  $\delta^{13}$ C values. The powders had been successively milled in 1 mm increments along the growth axis of the flowstone. The milled increments extended approximately 20 mm x 20 mm deep into the flowstone, parallel to the growth banding.

The masses of the samples were: 22.6 mg, 36.1 mg, 111.7 mg and 40.1 mg, generating signals of 213 mV, 490 mV, 1371 mV and 588 mV, respectively. The suitable analytical ranges for the IRMS are amplitudes between 1–8 k. The masses of the samples were insufficient to obtain a higher signal. Therefore, to provide sufficient signals with small samples, the injection volumes were increased to 20  $\mu$ l for the following analyses. The mass of preparation was set to 40 mg.



Figure 3.3 Ages of powders from Easter Cave Flowstone 1, acquired organic  $\delta^{13}$ C values and the ratios of amplitudes with mass. The fast growth periods displayed with the depth on the flowstone are shaded grey. The yellow circles display the U-Th ages, and the age correction only applies between 13 and 173 mm as these are the depths of the U/Th ages that bracket the main span of growth in ESF1.

Figure 3.3 represents the corrected U-Th ages, organic carbon isotope values and the ratios of amplitude to mass acquired based on the depth above the bottom of ESF1. It can be seen that the isotope values range from -26.4‰ VPDB to -23.5‰ VPDB. The overall standard deviation is 0.2‰ VPDB on repeated individual values. Ratios of amplitudes to masses range from 55 V/g to 160 V/g. The youngest group lies within the period of slow growth, and the other two groups are basically within a period of fast growth, except the last two acquired at 145 mm and 146 mm.

# 3.3.2.2 Calcite and Organic Carbon

Our three analysed groups provided organic  $\delta^{13}$ C values for the ages between 7.2 ka to present ( $\delta^{13}$ C = -26.0% VPDB ± 0.2%), 9.2 ka to 9.4 ka ( $\delta^{13}$ C = -26.0% VPDB ± 0.5%), and 10.6 ka to 11.2 ka ( $\delta^{13}$ C = -26.2% VPDB ± 0.2%). The organic carbon isotope values are consistent through time. Comparison of the calcite  $\delta^{13}$ C values acquired from the dual-inlet IRMS and the organic  $\delta^{13}$ C acquired from our LC-IRMS can be observed in Figure 3.3. It shows that three different spatial groups provide stable organic  $\delta^{13}$ C values regardless of time changes (overall  $\delta^{13}$ C = -26.1% VPDB ± 0.3%), compared to the relatively large variations in calcite  $\delta^{13}$ C. The three same time intervals as the organic groups provide calcite  $\delta^{13}$ C<sub>0mm-10mm</sub> = N/A,  $\delta^{13}$ C <sub>89mm-97mm</sub> = -8.2% VPDB ± 0.4%, and  $\delta^{13}$ C <sub>137mm-146mm</sub> = -10.5% VPDB ± 0.5%.



Figure 3.4 Comparison of calcite  $\delta^{13}$ C and organic  $\delta^{13}$ C based on the ages.

# 3.4.1 Method Development

The mechanism of this methodology is to oxidise the residual acid-soluble OM through liquid chromatography, and the reacted  $CO_2$  with the IRMS detector was captured by the IRMS detector to solely provide an OM  $\delta^{13}$ C value. As long as adequate oxidant and catalyst are provided through the LC progress, the method should be robust under various chemical conditions. Providing that more samples injected would be followed by the higher amplitudes signals, a decrease in the injection amount could help stabilize the whole procedure.

Comparing the different conditions used in the study, one major factor is recognised to determine  $\delta^{13}$ C values: injection volumes and oxidant of standards. When the injection volumes were set to 20 µl,  $\delta^{13}C_{20\mu l \text{ corrected}} = -24.2\%$  VPDB ± 0.3‰. As a comparison,  $\delta^{13}C_{5\mu l \text{ corrected}} = 23.3\%$  VPDB ± 0.3‰. With a further look at the cause of this, the difference in the amplitudes appears to be the dominating influence. The amplitude range for a 20 µl injection was 1.18–1.62 V, and that of a 5 µl injection was 0.12–0.41 V. Moreover, the ideal range of detection for the IRMS is 1–8 V. Thus, the instrumental parameters should be responsible for this shift. Other relatively large changes in chemical conditions merely lead to minor shifts in the calcite  $\delta^{13}$ C of organic matter, especially when the smallest samples are injected into the machine ( $\delta^{13}$ C = -22.0‰ VPDB ± 0.27‰).

Thus, this method could be demonstrated to be reliable for stalagmites under different preparation and instrumental settings, with a low average standard deviation (~0.2‰), and low sample consumption (~25 mg).

One problem with the flowstone analysis was reproducibility. Duplication of samples failed to provide a level of accuracy that matched the individual sample precision, as Figure 3.5 Replication using the same samples shows.



Figure 3.5 Replication using the same samples: organic  $\delta^{13}$ C values (a.) and ratios of amplitudes and masses (b.).

As the ratios of amplitudes and masses represent the amount of carbon injected into the mass detector, the OM amount in the two experiments was kept constant (see Figure 3.5 Replication using the same samples.b.). However, an overall mean increase of  $\delta^{13}$ C = 3.5‰ was observed for the duplicates (see Figure 3.5 Replication using the same samples.a.). Also, the mean standard deviation increased to 0.3‰ for one single sample comparing with 0.01‰ in the first series.

There are several hypotheses for this phenomenon.

1) Instrumental errors, including septa and tube contamination, syringe blockages and inner filter blockages. However, these are unlikely to occur for all repetitions. The acquired  $\delta^{13}$ C values for all the standards were relatively stable along the experiments (SD for  $\delta^{13}C_{USGS40-1st} = 0.5\%$  compared to  $\delta^{13}C_{USGS40-2nd} = 0.5\%$ ). As a former hypothesis suggests, the oxidant condition was sufficient to supply a full chemical reaction on the speleothem. The instrumental variation should not be listed as the primary target for future research using other samples.

2) Preparation problems, including inhomogeneity of sampling spots on the flowstone, changes in the components of OM, and preservation. The two former hypothesises were unlikely to occur with a constant  $\delta^{13}C = 3.5\%$  increment on all the duplicates. The powders were sealed with aluminium foil and stored in the lab for months before the start of the experiments. There is still a possibility that the powders were partly oxidated by air before the analyses, which could result in a positive trend on all of them. In addition, a notable difference between the two runs is that the powder in the

second run was dissolved by the 3 M phosphoric acid within 5 mins, which was twice as fast as the average duration for the first series.

The first series had a lower variability and lower mean  $\delta^{13}$ C. Both things suggest that the second series had a problem which led to increased variability and fractionation. As a consequence, the second series was considered inappropriate for analysis. Hence, the following discussion is based solely on the first series.

### 3.4.2 Paleoclimate Functions of ESF1

# 3.4.2.1 Surface Vegetation Changes

Through the formation of speleothem, there are several processes that affect  $\delta^{13}$ C: surface vegetation types, soil CO<sub>2</sub>, change in atmospheric pCO<sub>2</sub>,  $\delta^{13}$ C fractionation during calcite precipitation and contributions from in-cave sources of OM (e.g. microbes on the speleothem surface) (McDermott, 2004; Cacchio et al., 2004).The vegetation within the Augusta karst area contains tall (20–40 m) forests of karri (*Eucalyptus diversicolor* F. Muell.) on deep loams, with jarrah-marri (*E. marginata* Sm. *Corymbia calophylla* {Lindl.} K.D. Hill and L.A.S. Johnson) forests on leached sands (Beard, 1990). Other scrub plants on calcareous sands include *Agonis flexuosa* (Willd.) Sweet, *Banksia grandis* Willd., *E. angulosa* Schauer, and *Acacia* spp.. Thus, the majority of vegetation above Easter Cave is C3 plants.

Figure 3.3 shows the ages of powders from Easter Cave Flowstone 1, acquired organic  $\delta^{13}$ C values and the ratios of amplitudes with mass. The fast growth periods are shaded in grey colour. The organic  $\delta^{13}$ C remained stable across the early half of the Holocene, providing an overall  $\delta^{13}$ C = -26.0‰ VPDB <u>+</u> 0.3 ‰. Almost no variation

occurred across different groups ( $\delta^{13}C_{0mm-10mm} = -26.0\%$  VPDB  $\pm 0.2\%$ ,  $\delta^{13}C_{89mm-97mm} = -26.0\%$  VPDB  $\pm 0.5\%$ , and  $\delta^{13}C_{137mm-146mm} = -26.2\%$  VPDB  $\pm 0.2\%$ ) and across the growth period (28 samples in slow growth period with  $\delta^{13}C = -26.0\%$  VPDB  $\pm 0.3\%$ , compared to two samples in the fast growth period with  $\delta^{13}C = -26.2\%$  VPDB  $\pm 0.2\%$ ). Based on this, it can be proven that there was little change in the ratios of C3 and C4 plants across time across the early half of the Holocene. The vegetation ratios which led to the large variation in organic  $\delta^{13}C$  could be minimised under such circumstances. The shift occurring in calcite  $\delta^{13}C$  on the ESF1 (see Section 3.3.2.2) around 10 ka is demonstrated to be a non-vegetation-related phenomenon.

## 3.4.2.2 Paleoclimate

There are three fast growth periods appearing along the flowstone: 7.3–7.8 ka, 9.2–9.5 ka and 10.2–10.8 ka. Former paleoclimate studies have reported cooling events at 8.2 ka (Muller et al., 2008), 9.3 ka (Crombé, 2018; Fleitmann et al., 2008), and 10.8 ka (Imagawa et al., 2009). The 8.2 ka event was triggered by a meltwater pulse (MWP) into the North Atlantic and resultant reduction of the thermohaline circulation (THC). The 9.3 ka Before Present (BP) event was dated between 9300 and 9190 calendar years (cal) a BP, and has climatic anomaly patterns very similar to the 8.2 ka BP event. The Younger Dryas Cooling Event was recognised between 11–10 ka. However, the calcite  $\delta^{13}$ C could not provide sufficient support for the growth rate events, as carbon isotopes did not change synchronously. During all these cooling events of the northern hemisphere, the only possible response on this southern hemisphere flowstone was the growth during 10.2 ka to 10.8 ka, which provided a notable  $\delta^{13}$ C = -10.3‰ VPDB ± 0.6‰, compared to the overall  $\delta^{13}$ C = -9.6‰ VPDB ± 1.1‰. However, we could not

confirm its correlation with cooling events without further supplementary evidence. A more recent record based on overlapping sediment cores in Barker Swamp, southwestern Western Australia (Gouramanis, 2012) reconstructed a detailed history of evaporation/precipitation (E/P) after 8.7 ka. Researchers suggest there was increased E/P around 6.7 ka and 5.9 ka, respectively. Compared to Barker Swamp record, the calcite  $\delta^{13}$ C of ESF1 indicates a decreasing trend from 8.6 ka to 7.8 ka ( $\delta^{13}$ C from -7.2‰ VPDB to -11.8‰ VPDB), and 7.6 ka to 7.4 ka ( $\delta^{13}$ C from -7.4‰ VPDB to -9.8‰ VPDB), as shown in Figure 3.4. There was a gap between the two chronological records within the nearby regions. A further step to precisely assess the age of ESF1 at depths lower than 10 mm is required to test any linkages.

#### 3.4.2.3 Isotope Kinetics and Fractionation on the Flowstone

Fractionation factors occurring through the growth of flowstone would result in the non-equilibrium isotope kinetic for the organic matter and inorganic calcite carbon  $\delta^{18}$ O values. One importance character of non-equilibrium kinetic is the degassing of CO<sub>2</sub>. In specific regions, CO<sub>2</sub> degassing may be too fast to complete the CO<sub>2</sub> hydration-hydroxylation reactions buffering the oxygen isotopes (Mickler et al., 2006, 2015). It can be reflected within the ratios of  $\delta^{13}$ C to  $\delta^{18}$ O.

The scatter plot for calcite  $\delta^{18}$ O and  $\delta^{13}$ C displayed in Figure 3.6 represents the changes in their ratios over time. The distributions of calcite  $\delta^{18}$ O and calcite  $\delta^{13}$ C in different groups are almost parallel to each other. Different intercepts are the result of hydfrology or climate changes across time, while similar slopes indicate that they are still highly correlated. The fractionation phenomenon manifests itself by producing a

positive correlation between  $\delta^{18}$ O and  $\delta^{13}$ C calcite values, in this case, the overall slope is 1.38. This can be compared to the non-buffering slope of 0.52, and an equilibrium slope which will lead to infinity (Mickler, 2006). For reference, the series between 89– 97 mm and 137–146 mm displayed slopes of 1.56 and 1.11, respectively. The extent of the fractionation phenomenon could change over the large time scale due to several cooling events, leading to minor variations in the slopes. However, they are generally parallel to each other, suggesting that relatively consistent isotope fractionation occurred throughout the flowstone's growth.



Figure 3.6 Scatter distribution comparing calcite  $\delta^{18}$ O and calcite  $\delta^{13}$ C. The slope for all samples across the flowstone is 1.11, and that for group 89–97 mm is 1.38, while for 137–146 mm it is 1.56.

#### 3.5 Conclusion

This method has been proven to be reliable on the homogenised stalagmite, ignoring condition changes such as flow rates and concentrations of acid, and provides stable results for different mass amounts. The minimum consumption utilised in this study is 25 mg of calcite, with a low standard deviation (~0.2‰ VPDB) under constant preparation and instrumental conditions. The application onto ESF1 successfully reconstructs a constant ratio of C3 and C4 plants across the early half of the Holocene, with a mean  $\delta^{13}$ C = -26.0‰ VPDB + 0.3 ‰ for all the substances. Comparing to the calcite oxygen and carbon isotopes, the main driven force leading to the variations in calcite isotopes is recognised to be the fractionation.

This work has demonstrated a reconstruction of paleoenvironment through the combined use of organic and inorganic carbon isotopes onto the flowstone. However, two aspects of future work should be examined: methodology robustness improvement and application onto substances from sites with variations between C3 and C4 plants. Some unknown factors occurred during the second running of the duplicates, which requires further research. In future work, the calcite  $\delta^{13}$ C for the 175-185 mm group (< 8 ka) will be analysed to validate it with the local vegetation records. The intention is to synthesise  $\delta^{13}$ C data for modern-day native vegetation, and compare that to the most recent  $\delta^{13}$ C -OM from the flowstone. Secondly, the comparison between calcite  $\delta^{13}$ C and cores records published by Gouramanis et al. (2012) would be conducted to validate the links between sea levels and calcite  $\delta^{13}$ C with a similar regional context. The majority of vegetation in Augusta is C3 plants, thus

the organic carbon isotope values acquired from this region are less affected by the climate-driven C3-C4 plants ratio changes over 20 thousand years. Karst regions with adequate C4 angiosperms distribution like Rhenish Massif, Germany and Xishuangbanna Tropical Botanical Garden, southern Yunnan, China should be considered. We would also include wider geographical links, including the Australian Holocene records based on salt-disrupted sediment during the past 150 ka (Magee et al., 2004), peat core during the past 8 ka (Marx et al., 2009) and summarised pollen evidence during the past 6 ka (Shulmeister and Lees, 1995). After that, comparisons with other speleothem Holocene records in the Northern Hemisphere will be conducted, including US (Dorale et al., 1992; Harmon et al., 2004), UK (Roberts et al., 1998), Belgium (Verheyden et al., 2000) and China (Li et al., 2014).

#### CHAPTER 4 Conclusions

This thesis aimed to further develop our understanding of the responses of speleothems to surface variations such as short-term fire events and long-term climate patterns. Chapter 2 produced an innovative method of hydrographic analysis to recognise different stages of subsurface recharge. Based on this, the hydrological properties affected by changes to the surface soil due to the intense experimental fire were concluded to be increased preferential flows and decreased capillary flows. In addition, evaporated surface water preserved in the soil and volatilised vegetation-derived ashes were both identified in this study. In Chapter 3, a quick method of analysing organic carbon isotopes was proposed and demonstrated. Then, through comparison with calcite carbon isotopes, we proved that fractionation is the major influence on the changes in calcite  ${}^{13}C/{}^{12}C$  ratio over time, rather than changes in surface vegetation.

Both approaches demonstrate that in complex hydrogeological settings, the combined use of isotope analysis and hydrograph analysis can provide insights into local karst hydrology and groundwater processes. In the short term, it revealed the occurrence of increased preferential flows and decreased capillary flows. At the other end of the scale, an insight into the organic carbon isotope fractionation mechanism was obtained that is useful for paleoclimatic reconstructions. These improve the current understanding of the importance of surface environments to subsurface systems, enabling better forest management and providing possibilities to extend the paleo-fire histories further back in time. This study also highlights new directions for future cave-based geochemical research. In small time scales, fire was confirmed to have produced increased preferential flows and decreased capillary flows. However, how this influence translates to the largerscale underground water resource is still to be investigated. Organic carbon isotopes, as important signatures of C3 to C4 plant ratios, were stable throughout the growth period of ESF1 (approximately 20,000 years), which helps to understand the changes on calcite carbon isotopes in the absence of vegetation influences. However, more evidence, such as from more stalagmites lying under abundant C4 vegetation, are essential to achieving a full theory on the functions of organic carbon isotopes and their correlation with calcite carbon isotopes. Many puzzles remain in the supposedly 'well understood' relationship between the surface and subsurface.

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#### **APPENDIX 1**



Supplementary Figure 1.1. Temperature records from 26<sup>th</sup> May to 1<sup>st</sup> June 2016. The probe was buried 12 cm below the surface.



Supplementary Figure 1.2. Drip records from individual dripping sites



Supplementary Figure 1.3. Drip rates at each site comparing the responses to each infiltration event for both pre- and post-fire events. Log10 transformation is performed on the *y*-axis. Site numbers are indicated on each panel. Different recharge events at individual sites are shown in different colours.



Supplementary Figure 1.4. Time series for cations and anions before and after fire. Units for  $Ca^{2+}$ ,  $Fe^{2+}$ ,  $K^+$ ,  $Mg^{2+}$ ,  $Na^+$ , Si,  $SO_4^{2-}$ ,  $I^-$  and  $CI^-$  are given in mg/I, while Ba and Sr are in  $\mu$ g/I. The fire event is indicated by the yellow line.

Site	State	Statistic	Number	Range	Min	Max	Sum	Mean		RSD	Variance	Skewness		Kurtosis	
1	Pre	Mean	3031	17	4	21	39557	13	0	3	9	-1.5	0.1	11.7	0.2
		SD	2360	5	2	7	31232	2	0	1	8	3.2	0.1	24.6	0.2
	Post	Mean	4371	16	1	17	33945	8	0	3	12	0.5	0.0	-0.7	0.1
		SD	1690	3	0	3	19230	2	0	1	4	0.4	0.0	1.0	0.0
2	Pre	Mean	1277	1615	34	1648	316067	331	33	286	117157	1.9	0.2	7.4	0.3
		SD	1216	1316	46	1306	302187	269	80	196	153420	1.8	0.2	14.5	0.4
	Post	Mean	302	1908	58	1965	115770	977	151	613	538057	0.2	0.5	1.5	0.8
		SD	1052	1218	178	1256	326628	743	171	411	614171	1.5	0.3	5.1	0.6
3	Pre	Mean	649	1493	17	1510	325747	392	24	359	242005	0.3	0.2	6.6	0.4
		SD	656	1411	58	1403	415465	312	41	344	326179	2.7	0.2	14.1	0.3
	Post	Mean	56	2438	1	2439	32962	612	98	718	551958	1.3	0.3	0.7	0.6
		SD	6	537	0	537	13536	314	42	269	385963	0.6	0.0	1.9	0.0
4	Pre	Mean	859	555	21	576	28503	120	16	82	10345	9.3	0.3	204.8	0.5
		SD	738	349	34	347	40701	156	26	73	12250	10.9	0.3	314.3	0.7
	Post	Mean	640	520	11	531	38467	110	26	119	22437	2.3	0.3	8.2	0.5
		SD	625	328	16	321	30707	78	38	95	31679	1.3	0.4	9.8	0.5
5	Pre	Mean	843	246	7	252	60082	73	2	60	4718	1.9	0.1	5.0	0.2
		SD	213	96	11	105	64075	72	1	38	5323	1.7	0.0	8.3	0.0

Supplementary Table 1.1. Descriptive statistics on individual recharge events at every site pre- and post-fire.
	Post	Mean	743	563	8	571	95658	167	38	161	56687	1.4	0.3	2.3	0.6
		SD	576	494	7	498	110265	154	80	188	126878	1.0	0.3	5.0	0.7
6	Pre	Mean	18	1632	7	1639	11986	572	156	566	513032	0.7	0.7	-0.5	1.4
		SD	17	1411	6	1410	14455	504	146	469	805118	0.7	0.3	1.5	0.7
	Post	Mean	99	1879	7	1887	98894	927	58	595	434724	0.2	0.3	-1.0	0.5
		SD	24	772	12	770	43985	358	27	306	365987	0.5	0.0	0.6	0.1
7	Pre	Mean	1252	329	1	329	70551	15	9	46	13576	1.6	0.2	10.8	0.4
		SD	1832	581	1	580	92466	19	11	74	23816	2.2	0.3	14.5	0.5
	Post	Mean	747	1260	11	1260	134863	315	36	140	199484	2.2	0.1	15.3	0.2
		SD	952	1471	13	1469	221392	353	43	168	235920	2.5	0.1	19.9	0.3
11	Pre	Mean	79	1478	2	1481	39942	485	78	481	323095	1.0	0.4	0.3	0.7
		SD	73	841	2	841	46975	383	80	316	321623	0.6	0.2	1.6	0.3
	Post	Mean	65	1328	6	1334	43258	512	65	434	274403	0.7	0.4	-0.4	0.8
		SD	50	882	12	883	46527	373	56	299	287702	0.6	0.2	1.2	0.5

## APPENDIX 2

## Supplementary Table 2.1. Conditions and results of method development using the USGS-40 standard.

	Standard			Stalagmite						
Series	Amp. (mV)	Amp. $\delta$ <sup>13</sup> COffset(mV)(‰VPDB)(‰)		Amp. δ <sup>13</sup> C   (mV) (‰VPDB)		Final (‰VPDB)	Parameters			
1	6025.6	-24.6	-1.79	11787.9	-22.9	-24.69	Standard condition			
2	2941	-21.5	-4.89	17397.7	-22.0	-26.89	0.30% Sulfuric mobile phase (Phase C)			
3	6031.1	-25.3	-1.09	15214.7	-23.0	-24.09	$30\ \mu l/min$ flow rates for catalyst and oxidant			
4	5220	-25.5	-0.89	15230.6	-23.2	-24.09	1.5 M Catalyst+30 μl/min			
5	4486	-25.3	-1.09	3970.5	-22.1	-23.19	5 μl Injection			
6	5154.5	-25.2	-1.19	4084.8	-21.8	-22.99	5 μl + 30 μl/min			
7	4514.6	-25.0	-1.39	1217.1	-21.8	-23.19	5 $\mu l$ + 25 mg+1 M preparation acid + 30 $\mu l/min$			
8	4470	-24.9	-1.49	1220.2	-21.8	-23.29	5 $\mu l$ + 25 mg + 1 M preparation acid + 24 hr + 30 $\mu l/min$			
9	4639.8	-25.0	-1.39	1187.3	-21.9	-23.29	5 $\mu$ l + 25 mg + 2 M preparation + 30 $\mu$ l/min			
10	4679	-24.9	-1.49	1188	-22.2	-23.69	5 $\mu$ l + 25 mg + 2 M preparation + 24 hr			
11	5995.5	-25.4	-0.99	16161.6	-23.1	-24.09	Phase C + 30 µl/min			

12	4542.1	-25.2	-1.19	3999.7	-22.1	-23.29	Phase C + 5 µl
13	4754.7	-25.6	-0.79	16101.4	-23.0	-23.79	Phase C + 1.5 M catalyst + 30 $\mu$ l /min
14	4447.8	-25.1	-1.29	1199.5	-22.0	-23.29	Phase C + 5 $\mu l$ + 25 mg + 1 M preparation + 30 $\mu l/min$
15	4690	-24.9	-1.49	1200.5	-21.5	-22.99	Phase C + 5 $\mu l$ + 25 mg + 1 M preparation + 24 hr + 30 $\mu l/min$
16	4462.6	-25.0	-1.39	1185.8	-22.2	-23.59	Phase C + 5 $\mu l$ + 25 mg + 2 M preparation + 30 $\mu l/min$
17	4602.7	-25.0	-1.39	1172.8	-22.4	-23.79	Phase C + 5 $\mu l$ + 25 mg + 2 M preparation + 24 hr + 30 $\mu l/min$