Tracing local hydrology and water source use of *Eucalyptus largiflorens*, on the Calperum Floodplain, using Strontium, Oxygen and Deuterium Isotopes

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TRACING LOCAL HYDROLOGY AND WATER SOURCE USE OF *EUCALYPTUS LARGIFLORENS*, ON THE CALPERUM FLOODPLAIN, USING STRONTIUM, OXYGEN AND DEUTERIUM ISOTOPES

TRACING LOCAL WATER SOURCE USE OF *EUCALYPTUS LARGIFLORENS*

ABSTRACT

Black Box trees (*Eucalyptus largiflorens*) across the Murray-Darling Basin are in critical condition due to high groundwater salinity and infrequent natural flooding. Geochemical tracers such as radiogenic Strontium ($^{87}Sr/^{86}Sr$), Oxygen-18 ($\delta^{18}O$) and Deuterium (δD) are considered useful applications in the understanding of catchment hydrology and plant water use, and in this study, $^{87}Sr/^{86}Sr$, $\delta^{18}O$ and δD isotopes were used accordingly to better comprehend local hydrology and water use behaviour patterns of Black Box trees on the Calperum Floodplain, South Australia. Investigations were achieved by sampling and analysing local surface waters (Lake Merreti, Lake Clover, and River Murray), groundwater, soils (1.5 m depth) and plant material (stem water, and leaves) from two separate sites, north (Site 1) and south (Site 4). Considering the local hydrology, Lake Clover was composed of evaporated rainwater, while Lake Merreti was a relative mix of both evaporated rainwater and river water. Additionally, local rainfall sources appeared to vary overtime. Furthermore, groundwater showed no close relationship with rain water suggesting an alternative recharge source such as river water or remnant paleo-water. In terms of water use, linear mixing models using soil $^{87}Sr/^{86}Sr$, leaf $^{87}Sr/^{86}Sr$ and stem water $\delta^{18}O$ inputs showed that Site 1 trees, on average, were predominately using rainwater (77%, 77% & 67%), while Site 4 trees used both rainwater (16%, 32% & 42%) and saline groundwater (70%, 62% & 58%), regardless of nearby lakes and streams. These findings have implications for future monitoring, and the management of outer floodplain Black Box populations that are unable to receive natural flooding inundation.

KEYWORDS

Black Box trees, hydrological tracing, strontium isotopes, oxygen isotopes, hydrogen

isotopes, Murray Darling Basin

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1. INTRODUCTION

Landscape and climate processes have significant implications for local hydrology and water accessibility, influencing ecology, health, and species distributions (Bendix & Hupp, 2000). For instance, human-induced climate change, as well as overpopulation and water scarcity is currently threatening global ecosystems that are reliant on available water (Garg, 2016; Pachauri & Meyer, 2015). It follows that understanding ecological and landscape processes is, therefore, vital for the ongoing management, survival, and prosperity of natural resources, both locally and globally**.** Australia, a predominantly arid continent, relies on available water for agriculture, water consumption, natural vegetation, and forestry (Spies & Dandy, 2012). Thus there is incentive to elucidate the relationship between the hydrological cycle and ecological processes to solve future water challenges.

Subsequent to the millennium drought (2000-2009), Australia enacted legislation (MDBP, 2012) which sought to regulate environmental flows (2,750 G) throughout the Murray-Darling Basin to prevent future drought impacts and natural vegetation loss (MDBA, 2018: Water Act, 2007). Allocated flows have been used to benefit Ramsar protected wetlands such as the Calperum Floodplain, South Australia, helping to manage the impacts of drought and high groundwater salinity (CSIRO, 2009; DWLBC, 2004). However, continued local management requires a deeper understanding of the local hydrology, water source mixing and water uptake behaviour to help prioritise the health and survival of selected vegetation populations (DEH, 2009a).

One species that has been identified as particularly threatened is the Black Box (*Eucalyptus largiflorens*) for which significant population health decline has been recorded in recent decades (George, Walker & Lewis, 2005; Moxham, Duncan & Moloney, 2017). Central to the debate around local water management issues is the uncertainty around the source of water consumed by disparate Black Box tree populations. Some literature has

suggested the trees are opportunistic consumers relying on rain and groundwater during inter-flooding periods (Jolly, & Walker, 1996). Other studies have shown that Black Box water uptake is spatially dependent on the recharge of deep soils by nearby lakes and creeks (Holland et al., 2006). Deciphering the differences in spatial dependency of water use by Black Box trees on the Calperum Floodplain is therefore crucial.

Geochemical isotopes, such as oxygen ($\delta^{18}O$), hydrogen (δD), and radiogenic strontium ($^{87}Sr/^{86}Sr$) are commonly used to understand the hydrological cycle and understand relationships between plants and soil (Capo, Stewart & Chadwick, 1998; Gat, 1996). Previously, $\delta^{18}O$ and δD have been used to trace water uptake source preference to help understand Black Box tree water use behaviour (Thorburn & Walker, 1993b). Currently, there has been no studies that have utilized both $^{87}Sr/^{86}Sr$, δD and $\delta^{18}O$ in understanding Black Box tree water use.

In this study, $\delta^{18}O$, δD, and $^{87}Sr/^{86}Sr$ was used to understand local hydrology and water source uptake preferences of Black Box trees on the Calperum Floodplain. It was expected that surface waters and groundwater would demonstrate distinct isotopic signatures. Furthermore, it was expected that Black Box was likely to show dependency on rainfall and groundwater; however, water source use was thought to also vary spatially and be influenced by the close proximity of rivers and lakes. Therefore, the sampling area was separated into two main sites, north and south, and assessed as separate systems. Each site, accompanied by isotopic data from surface and groundwater, was evaluated against the isotopic composition of soils, stems, and leaves to help draw conclusions about the water uptake source preferences of each Black Box population.

2. BACKGROUND

2.1. Calperum Floodplain

Climatically semi-arid, the Calperum Flooplain (34°02'43"S 140°42'52"E) is predominately composed of vegetation consisting of River Red Gum (*Eucalyptus camaldulensis*), Black Box (*Eucalyptus largiflorens*), River Cooba (*Acacia stenophylla*) and Lignum (*Muehlenbeckia florulenta*) (DEH, 2009b). Major tributaries in the area include Lake Merreti, Lake Clover, Lake Woolpool, Ral-Ral creek, Hunchee Creek and the River Murray. The floodplain experiences total rainfall averages of up to 260 mm per annum and, since 1963, rainfall has been reported to range from 86 mm to 556 mm (BOM, 2018). Notably, satellite imagery and modelling shows that the floodplain boundary follows the remnant extent of the 1956 flood event (Bloss, Montazeri & Eckert, 2015). The Calperum Floodplain requires 150,000 ML/day of water to reach 1956 levels and has historically occurred only 5-7 times between 1895 and 2009 (MDBA, 2012)**.** Groundwater bore depth, taken from 91 bores across the floodplain remains relatively homogenous at an average of 8.7 m (WaterConnect, 2018; 1991-2009). Measured groundwater salinity reaches 30-40 PSU[1] (Harper, 2007) with an average of 23 PSU[2] across the floodplain (WaterConnect, 2018; 2002-2011). Historically, the floodplain was formed from the incision of the River Murray, and the deposition of two ancient alluvial formations (DEH, 2009b). These two formations, composed of sand (Monoman Formation; depth >5 m) and clay (Coonambidgal Formation; depth 2-5 m), influence regional hydrology by restricting water infiltration and keeping groundwater as an unconfined aquifer (Figure 2). Surface soils exist as two broad groups, self-mulching cracking clays (fine clay) and crusty red duplex (red clay), and at depth consist of inter-bedded layers of clay and sand, and silt dominant surfaces (DEH, 2009b). Disturbed

[1] Converted from EC (mS/cm)
[2] Converted from EC (uS/cm)

floodplain surface soils and established trees provide water infiltration points for natural flooding, artificial flow inundation and rainfall events, helping to reduce groundwater salinity and recharging the local water table (Holland, Overton & Walker, 2004).

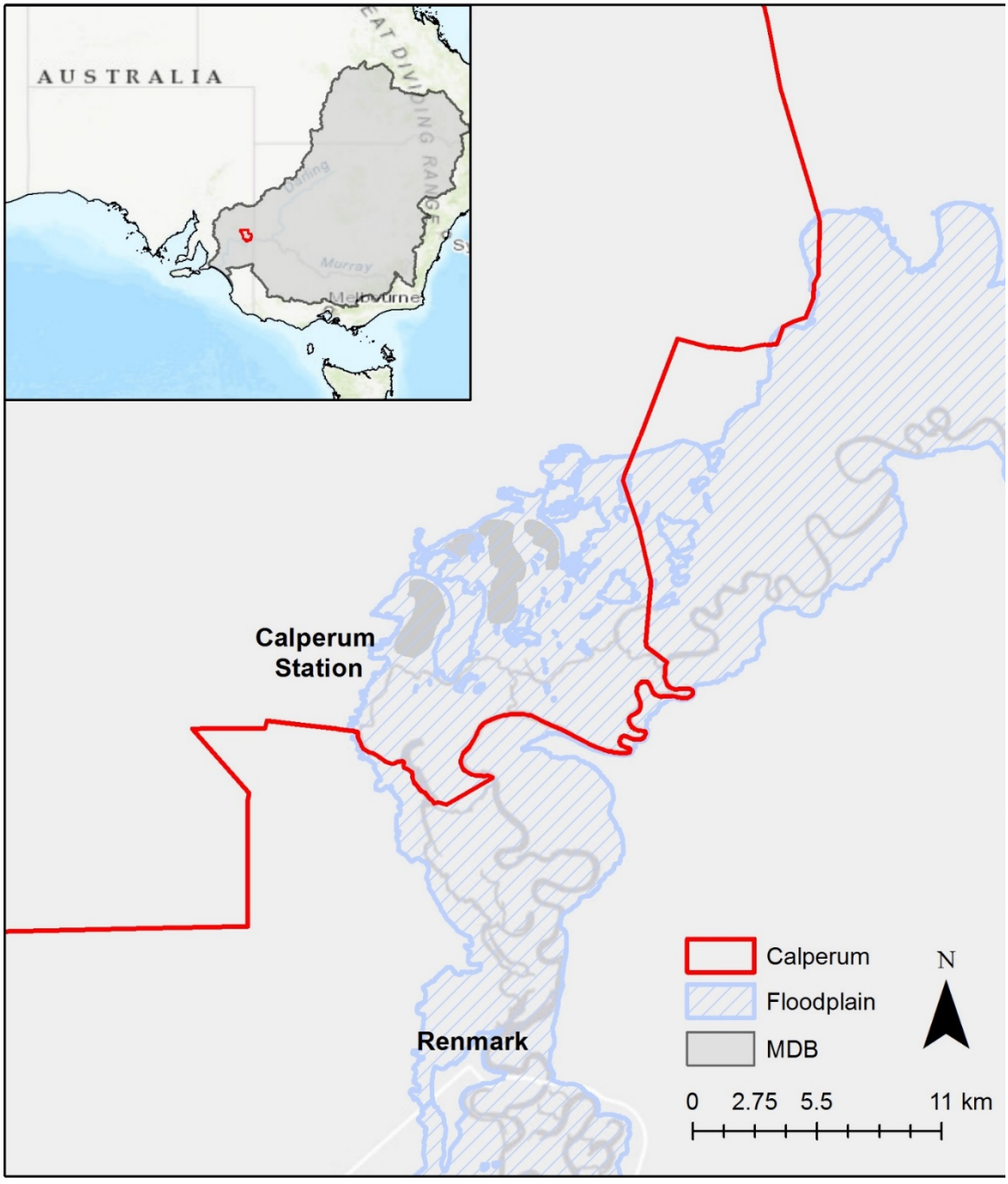


Figure 1 - A map showing the extent of the Murray-Darling Basin (see inset map) in relation to Calperum and the Calperum Floodplain. MDB = Murray-Darling Basin.

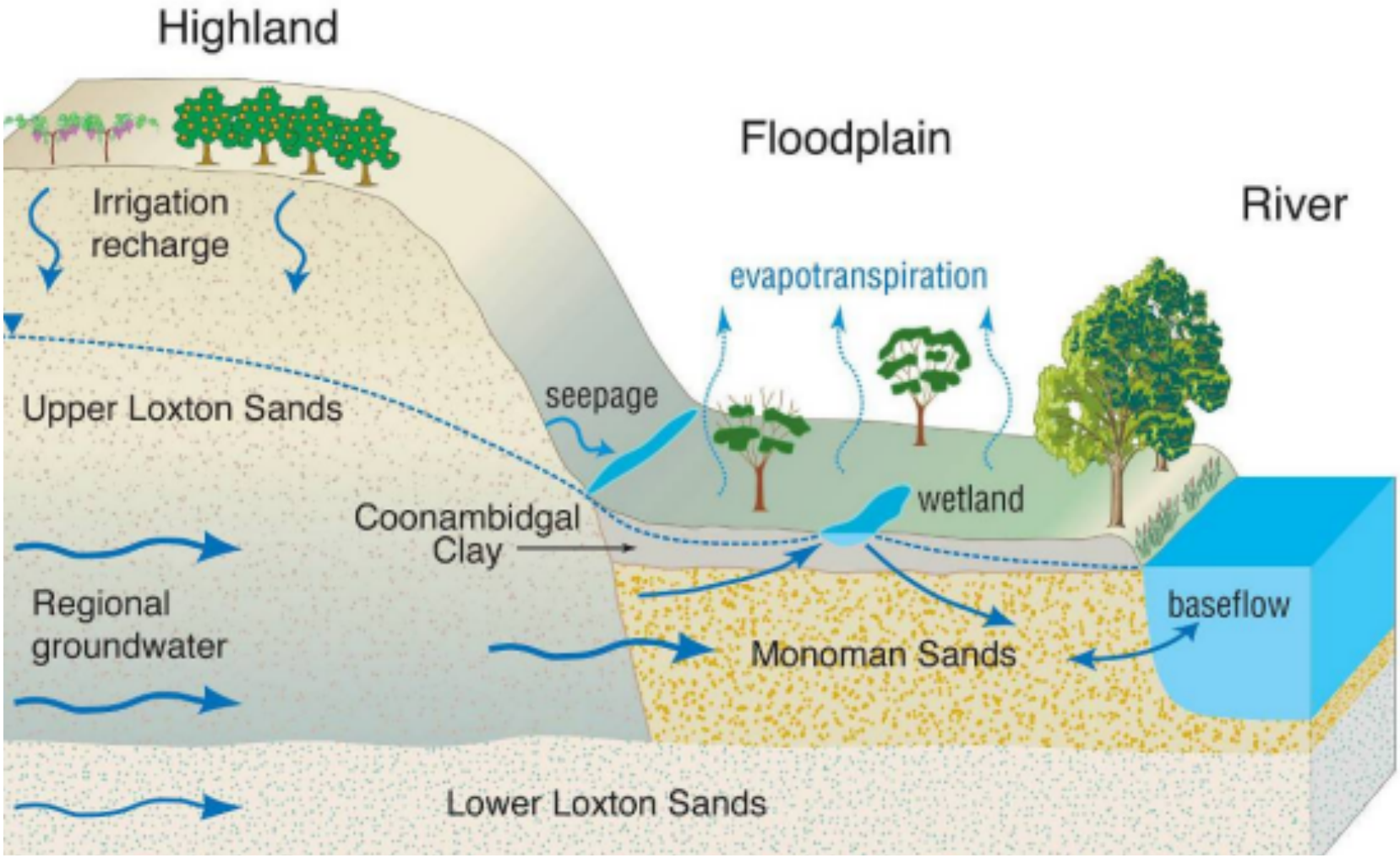


Figure 2 - Conceptual diagram showing groundwater flow and associated stratigraphy of floodplains (e.g. Calperum Floodplain) near the River Murray (Holland, Overton & Walker, 2004).

2.2. Black Box (Eucalyptus largiflorens)

Black Box trees (Figure 3) tend to form mainly open woodland forests and are known to be considerably drought and salinity tolerant, surviving in highly water depleted environments (Cunningham et al., 2011; Jolly et al., 1992). Found commonly on clay-rich arid/semi-arid floodplains, Black Box tree communities rely on episodic flooding events to help freshen groundwater stores, reduce groundwater salinity and provide necessary water supplies to new growth and seedlings (Agriculture Victoria, 2018; McEwan, Jolly & Holland, 2006). It has been estimated that Black Box populations require a flooding inundation event every 8 to 10 years to maintain population health (George, 2004). However, in between flooding events, Black Box trees utilize groundwater and rain when flood water is depleted (Jolly & Walker, 1996). Flooding events are unpredictable and rainfall varies substantially and this, in part, might explain why Black Box trees are opportunistic water consumers and switch between different water sources (Holland et al., 2006). Although adaptable to inter-flooding periods, groundwater is inaccessible to Black Box trees when water salinity levels are more than 25.5 PSU[3] which can cause major dieback when coinciding with times of drought and insufficient flows (DWLBC, 2004; Thorburn, Hatton & Walker, 1993a). This is a concern since dieback of Black Box trees can result in the loss of refuges for endemic fauna (Rogers & Ralph, 2011). Additionally, the lack of future established trees could also reduce infiltration points for water during flood inundation, reducing aquifer recharge (Bramley, Hutson & Tyerman, 2003) and increasing potential salinity levels further**.** Coupled with ongoing risks from climate change and water use, increasing inaccessibility of available water sources could continue to strain Black Box populations beyond the tolerance threshold for drought and salinity.

[3] Converted from EC (mS/cm)

Figure 3 - Photo of a Black Box tree taken infield on Calperum Floodplain. Credit: Alexander Harland

2.3. Geochemical Tracers

Oxygen (^{16}O, ^{17}O, ^{18}O) and hydrogen (^{1}H, D, ^{3}H) isotopes, both strongly correlated in water, vary throughout the hydrological cycle. Generally, oxygen and hydrogen isotopes are used because they are reflective of hydrological and meteorological processes. For example, vapour clouds sourced from evaporated ocean water shows a preference of lighter oxygen and hydrogen isotopes ($^{16}O/^{1}H$) to enter a vapour phase**.** Alternatively, enrichment occurs when water precipitates as rain due to the preferential inclusion of heavier isotopes during condensation ($^{18}O/^{2}H$) (Gat, 1996). In stable isotope geochemistry, oxygen-18 (^{18}O) and deuterium (D) isotopes are used to characterize temporal and spatial precipitation and evaporation in catchment hydrology, typically being denoted as a delta (δ) value and

standardized against mean ocean water (V-SMOW; Gröning & Fröhlich, 1999; Eq. 1.1). For instance, $\delta^{18}O$ and δD values of shallow groundwater are reflective of local precipitation (Gat & Tzur, 1967). Rivers, depending on the precipitation source, tends to deviate from local precipitation due to evaporation and external water sources, resulting in higher $\delta^{18}O$ and δD values. Similarly, lakes are also influenced by evaporation and source mixing (Henderson & Shuman, 2010). In lake catchments, positive $\delta^{18}O$ and δD values are exaggerated further when disconnected from external water source inputs and reside in highly evaporative environments (e.g. arid climates) (Horton et al., 2016). $\delta^{18}O$ and δD values are also known to be modified by biological activity (Gautam et al., 2017). In summary, $\delta^{18}O$ and δD, therefore, can and have been used to trace water origins, biological activity and water source mixing based on measured water sources in the environment.

$$\delta D = \frac{(^{1}H/^{2}H)sample}{(^{1}H/^{2}H)VSMOW} - 1 \times 1000 \text{ ‰} \quad \text{(Eq. 1.1a)}$$

$$\delta^{18}O = \frac{(^{18}O/^{16}O)sample}{(^{18}O/^{16}O)VSMOW} - 1 \times 1000 \text{ ‰} \quad \text{(Eq. 1.1b)}$$

Unlike $\delta^{18}O$ and δD, radiogenic strontium ($^{87}Sr/^{86}Sr$) does not appear to extensively fractionate under temperature and mass differences, due, in part, to the large ionic radius of the strontium (Sr) atom (Flockhart et al., 2015). An alkali earth metal, radiogenic ^{87}Sr isotopes are mobilised by the weathering of bedrock which is a product of radioactive decay of rubidium 87 (^{87}Rb). Landscape processes that interact with bedrock, dust, and soils, incorporate these weathered Sr ions which ultimately change the mass ratios of $^{87}Sr/^{86}Sr$. Consequently, $^{87}Sr/^{86}Sr$ ratios in water are dependent on factors such as residence time and

flow-path, and differ depending on the character of their water source origins (e.g. river, groundwater, lake), and underlying bedrock (Capo, Stewart & Chadwick, 1998).

Multi-isotope studies have used $^{87}Sr/^{86}Sr$, in conjunction with $\delta^{18}O$ and δD, to constrain environmental variables. For example, Britton (2010) used $\delta^{18}O$ to constrain climatic seasonality and $^{87}Sr/^{86}Sr$ to determine geographical regions to elucidate faunal migrations. Furthermore, a study using δD and $^{87}Sr/^{86}Sr$ to determine the origin of migratory birds found predictive modelling increased from 40% to 73% when using both isotopes (Sellick et al., 2009). Importantly, both radiogenic strontium and $\delta^{18}O$ and δD have been used as inputs for geostatistical models (isoscapes) to help predicted isotope patterns and variations in the environment**.** Previously, isoscapes, in conjunction with $\delta^{18}O$ and δD, have provided a means to understand continental precipitation variation, catchment hydrology, and water source mixing (Bowen & Good, 2015; Terzer, et al., 2013). Similarly, $^{87}Sr/^{86}Sr$ isoscapes have also been used to inform studies on human migration, criminal forensics and geological provenancing (Bataille, & Bowen, 2012; Laffoon et al., 2017; West et al., 2009).

2.4. Plant Isotope Ecology

Plants primarily derive their water and nutrients from the leaf canopy and/or from soils via their roots which are then transported up through the plant vascular xylem tissue by cohesion-tension forces (Steudle, 2001; Patrick, 2012). Water and nutrients are then incorporated into plant vascular tissues and cellulose, and used for photosynthesis and plant growth (Epstein, Thompson & Yapp, 1977; Leigh, 2016; Lopez & Barclay, 2016; Welch, 1995). Nutrient ions and water within plants have been useful in characterizing stable isotope signatures across different ecosystems and their functions (Dawson et al., 2002). Water has a constant presence in the xylem tissue and demonstrates no isotopic fractionation between soil water and the roots, therefore making it ideal for unravelling the relationship of water

availability, sourcing, and plant water uptake using $\delta^{18}O$ and δD (Bertrand et al., 2012; Berry, Hughes & Smith, 2014; Dawson & Ehleringer, 1993; Nie et al., 2012; Snyder & Williams, 2000; Thorburn, Walker & Brunel, 1993b; Thorburn & Walker, 1994; Wei et al., 2012). Additionally, bioavailable $^{87}Sr/^{86}Sr$, when mobile in soils and regolith, is incorporated into the wood, leaves, and stems of plants during growth, and has been applied to provenancing studies regarding plant soil uptake and ecological communities (Berg, 1995; Poszwa et al., 2004).

3. METHODS

3.1. Field Sampling

Surface waters, groundwater, soil, and organic material were collected on the 15th and 16th of May 2018 (Figure 4). Rainwater was collected, using a rain collector (Figure 5), on the 26th of August after being infield for 3 months. Two purpose built rain samplers were used, one bottle acid cleaned for Sr isotope analysis and other loaded with 5 ml paraffin oil to prevent evaporation for $\delta^{18}O$ and δD. Each sampler bottle was replaced every month for 3 months from May 16th to August 16th. River water was sampled along the northern River Murray bank at three different locations. Lake water was sampled from the shores of Lake Clover and Lake Merreti at locations separated by ~ 300-500 m. Groundwater was pumped and extracted from 3 different bore holes, within a 1.5 km^2 area, between Lake Clover and Lake Merreti. Samples were pumped out three times before sampling to limit surface fractionation effects**.** Lake Clover, Lake Merreti, River Murray and groundwater samples were measured for water quality variables, using a HANNA HI 98194 multiparameter, namely salinity (PSU), total dissolved solids (ppmTDS), conductivity (dS/m), pH, temperature (°C), and resistivity (mΩ /cm). Black Box tree stem and leaf samples were cut from 5 trees at two different sites using secateurs. Leaf samples were stored in paper bags

while stems were stored in sealable zip-lock bags. To represent a bulk average, leaves and stems, from each tree were collected from multiple points in the canopy. Additionally, soil cores, using a hand auger, were also taken at each site at 15 cm intervals for a total of 1.5 m, and the soil was then stored in zip-lock bags. After sampling, soil, water and stem samples were stored in either a refrigerator(s) below <4 C or a dry location to prevent fractionation and dust contamination. All samples were labelled and transported back to the lab within 1-2 days and kept in storage for 3-4 months before being analysed.

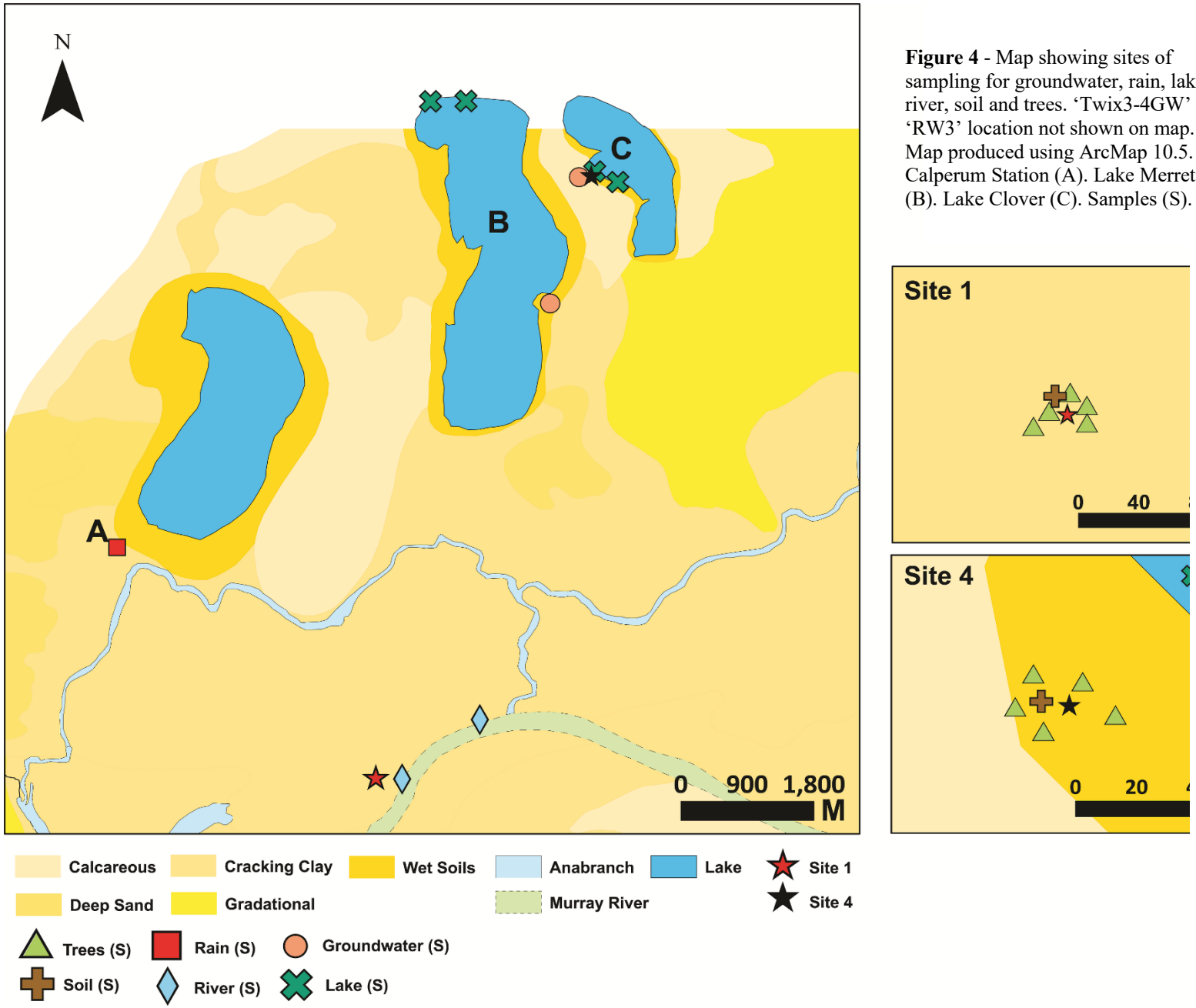


Figure 4 - Map showing sites of sampling for groundwater, rain, lake, river, soil and trees. 'Twix3-4GW' and 'RW3' location not shown on map. Map produced using ArcMap 10.5. Calperum Station (A). Lake Merreti (B). Lake Clover (C). Samples (S).

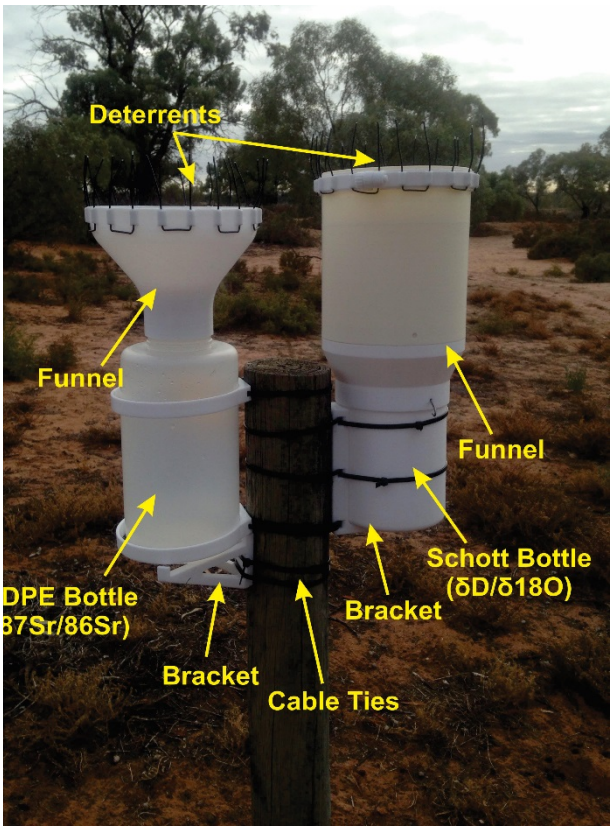


Figure 5 - Constructed rainwater collectors for $^{87}Sr/^{86}Sr$ (right) and $\delta^{18}O/\delta D$ (left) as seen infield. Schott bottle is obscured by bracket and is not seen here. Funnel entrances were physically filtered using detachable slitted-plugs to limit sediment influx. All components except for the bottles, deterrents and cable ties were 3D printed. Deterrents were placed on rim of funnel to prevent perching birds.

3.2. Isotope Analysis

3.2.1. $\delta^{18}O$ & δD

Preparation for each water source sample was limited. However, due to high total dissolved sediments (TDS) values, lake water had to be centrifuged post-collection to separate water from dissolved solids to meet analysis standards. A cryogenic distillation method was employed to extract water from soil and stem material adapted from Yang & Zhao (2010) (Figure 6). Stem, rather than leaf material, was chosen to avoid known leaf

water fractionation effects (Song et al., 2015). Extraction procedure was done over the course of 4 days at a temperature of 100 °C and pressure of 80-85 kpa. Due to the limited amount of water extracted from stem and soil via cryogenic distillation, samples were diluted with a known mass of deionized water (DI) prior to analysis. Dilution mixes, post analysis, were corrected for using a mass-balance equation (Eq. 1.2).

$$\delta_s = \delta_{s+d} - (m_s\delta_d)/m_d \qquad \text{(Eq. 1.2)}$$

δ_s being the unknown isotope sample value, δ_{s+d} as the diluted mixture, m_s as the mass of the extracted sample, δ_d as the known isotope value for DI, and m_d as the known mass of DI. DI-tap water diluted mixes, pure DI and pure tap water samples were prepared and also analysed, as controls to verify the validity of mass balance corrections. Samples, after preparation, were then analysed using Picarro L2130-i Isotopic Water Analyser at Flinders' Analytical laboratory. All extracted and collected samples were first filtered, to reduce contaminants, and then transferred into 2 mL vials. Each Picarro analysis was accompanied by an ongoing set of laboratory standards (desalinated water, bottled water and rainwater) and a 'dummy' sample as test comparisons.

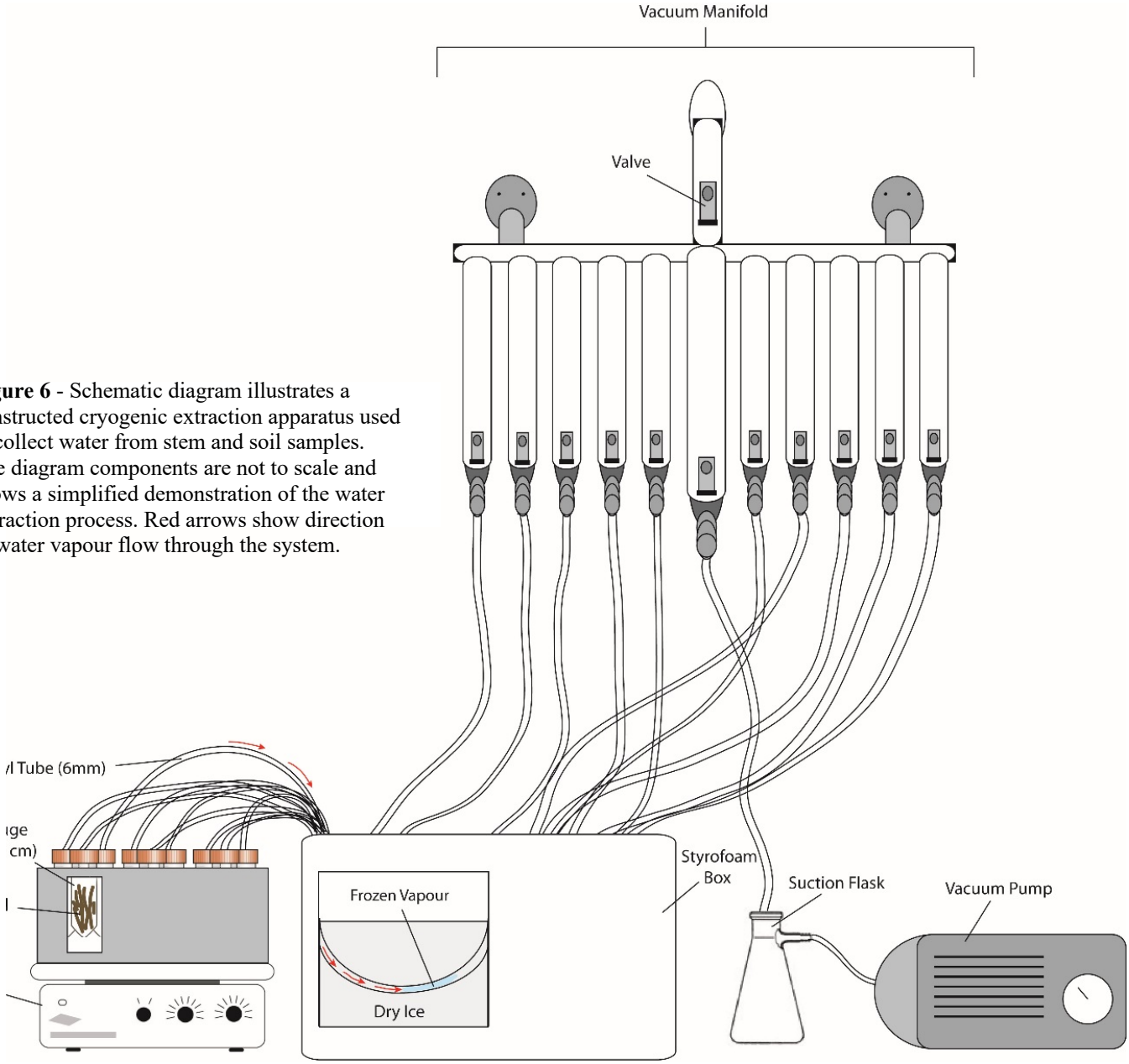


Figure 6 - Schematic diagram illustrates a constructed cryogenic extraction apparatus used to collect water from stem and soil samples. The diagram components are not to scale and shows a simplified demonstration of the water extraction process. Red arrows show direction of water vapour flow through the system.

3.2.2. Strontium ($^{87}Sr/^{86}Sr$)

All collected respective samples were prepared and subjected to a $^{87}Sr/^{86}Sr$ chromatographic column procedure (Romaniello et al., 2015; adapted) before being analysed using an Isotopx Phoenix Thermal Ionization Mass Spectrometer (TIMS). Three separate TIMS analyses were conducted and each run was accompanied by a TIMS standard, sample

standard and procedural blank(s). Procedural blanks were spiked with a known $^{87}Sr/^{86}Sr$ value and weighed to determine contamination, post-analysis, during preparation. Leaves, as opposed to stems, were chosen for $^{87}Sr/^{86}Sr$ analysis based on the assumption that they were representative of recent growth periods and water use. Prior to analysis, leaf samples were prepared and reduced to ash in an oven at 1050 °C. Soil samples, following a modified procedure from Reeuwijk (2002), were prepared and mixed in a solution of ammonium acetate to liberate bioavailable $^{87}Sr/^{86}Sr$ ions from soil exchangeable sites. Finally, because of the lack of organic and local regolith sample standards, seawater (IAPSO[4]) was chosen as the sample standard for all analysis runs and sample types.

3.3. Mixing Models

Isotope data-sets were analysed using a three-component linear mixing model (Phillips, Newsome & Gregg, 2005; Eq. 1.3) to determine, in comparison with analysed water sources (endmembers), the relative proportional isotope mass included in measured soils and organic materials. One model was used for each site.

$$\delta_m = \delta_a f_a + \delta_b f_b + \delta_c f_c \quad \text{(Eq. 1.3a)}$$

$$1 = f_a + f_b + f_c \quad \text{(Eq. 1.3b)}$$

Water source isotopes endmembers are denoted as δ_{abc}. Total mixture values (δ_m) were based on soil and leaf isotope data. Fractions of endmembers (f_{abc}) were determined and set to produce results within 4-5 decimal places of original soil and stem/leaf isotope totals (δ_m). Results were then plotted as ternary plots, using R package ggtern (Hamilton, 2018), to show

[4] See Appendix B

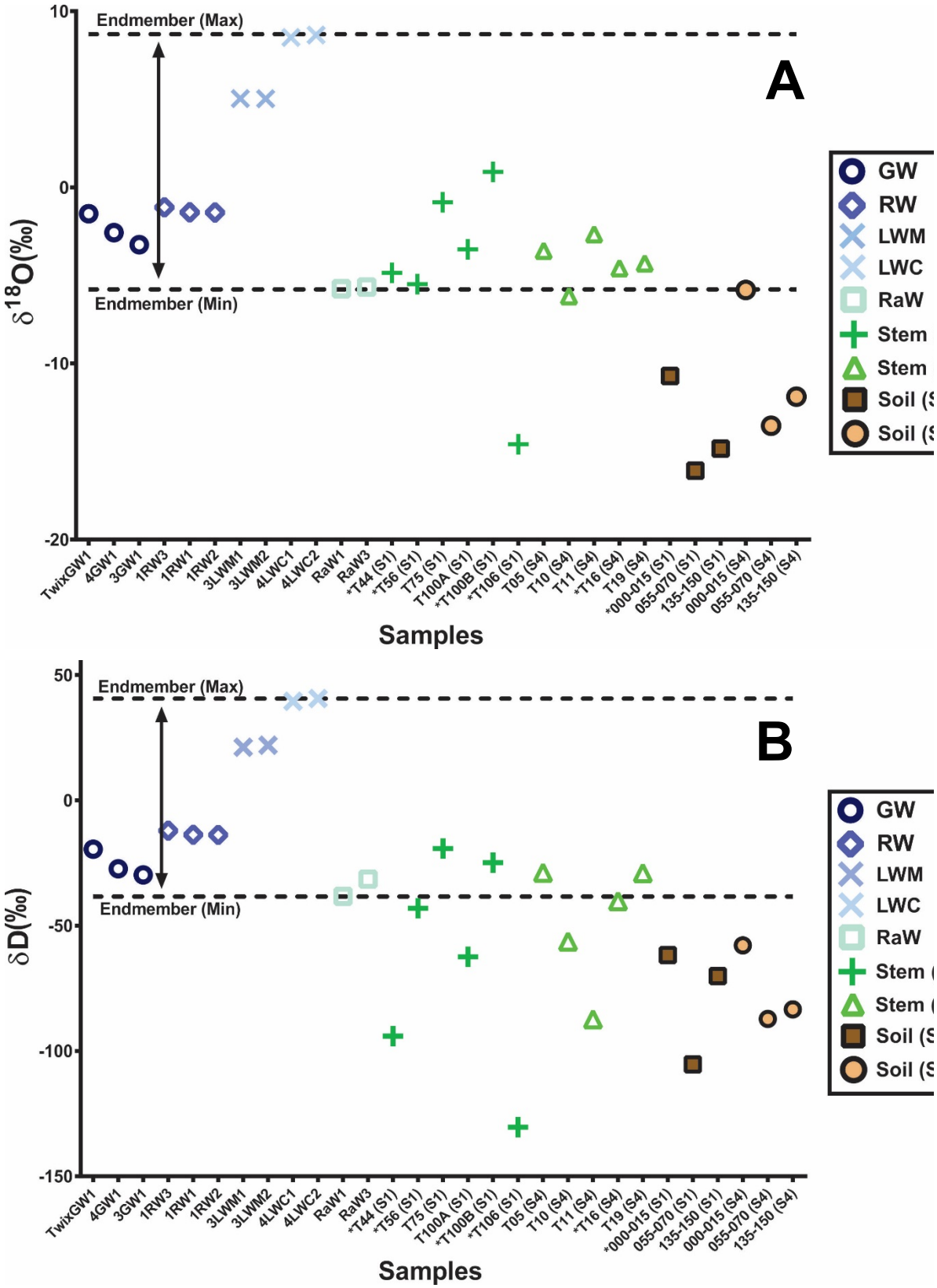
fractional inputs (f_{abc}) against a total percentage of 100.

3. RESULTS

3.1. $\delta^{18}O$ & δD

3.1.1. Surface and Groundwater

Measured $\delta^{18}O$ and δD values for surface waters and groundwater varied distinctly (Figure 7a & 7b). Collectively, groundwater (GW) sample values averaged 3.44 ‰ for $\delta^{18}O$, and -25.50 ‰ for δD. Only 2 out of 3 rain (RaW) samples, due to insufficient collection, were analysed, showing values of -5.76 ‰ and -5.66 ‰ ($\delta^{18}O$), and -31.42 ‰ and -38.33 ‰ (δD). Both lakes (LW), Lake Merreti (LWM) and Lake Clover (LWC), showed difference between average values of 5.04 ‰ (LWM) and 8.58 ‰ (LWC) for ($\delta^{18}O$), and 21.6 ‰ (LWM) to 40.04 ‰ (LWC) for δD. GW, RaW and RW clustered closest together with average values being -1.33 ‰ (RW), -5.71 ‰ (RaW) and -3.44 ‰ (GW) for $\delta^{18}O$, and -13.20 ‰ (RW), - 34.88 ‰ (RaW) and -25.50 ‰ (GW) for δD. LW sources, isotopically, sat the most distinctly separate from GW, RW and RaW with no overlap between values. Furthermore, $\delta^{18}O$ showed that there was a slight overlap between GW and RaW, and overlap between GW, RaW and RW for δD.


A
Endmember (Max)
Endmember (Min)
δ18O(‰)
10
0
-10
-20
GW
RW
LWM
LWC
RaW
Stem
Stem
Soil (S
Soil (S
TwixGW1
4GW1
3GW1
1RW3
1RW1
1RW2
3LWM1
3LWM2
4LWC1
4LWC2
RaW1
RaW3
*T44 (S1)
*T56 (S1)
T75 (S1)
T100A (S1)
*T100B (S1)
*T106 (S1)
T05 (S4)
T10 (S4)
T11 (S4)
*T16 (S4)
T19 (S4)
*000-015 (S1)
055-070 (S1)
135-150 (S1)
000-015 (S4)
055-070 (S4)
135-150 (S4)
Samples
B
Endmember (Max)
Endmember (Min)
δD(‰)
50
0
-50
-100
-150
GW
RW
LWM
LWC
RaW
Stem (
Stem (
Soil (S
Soil (S
TwixGW1
4GW1
3GW1
1RW3
1RW1
1RW2
3LWM1
3LWM2
4LWC1
4LWC2
RaW1
RaW3
*T44 (S1)
*T56 (S1)
T75 (S1)
T100A (S1)
*T100B (S1)
*T106 (S1)
T05 (S4)
T10 (S4)
T11 (S4)
*T16 (S4)
T19 (S4)
*000-015 (S1)
055-070 (S1)
135-150 (S1)
000-015 (S4)
055-070 (S4)
135-150 (S4)
Samples

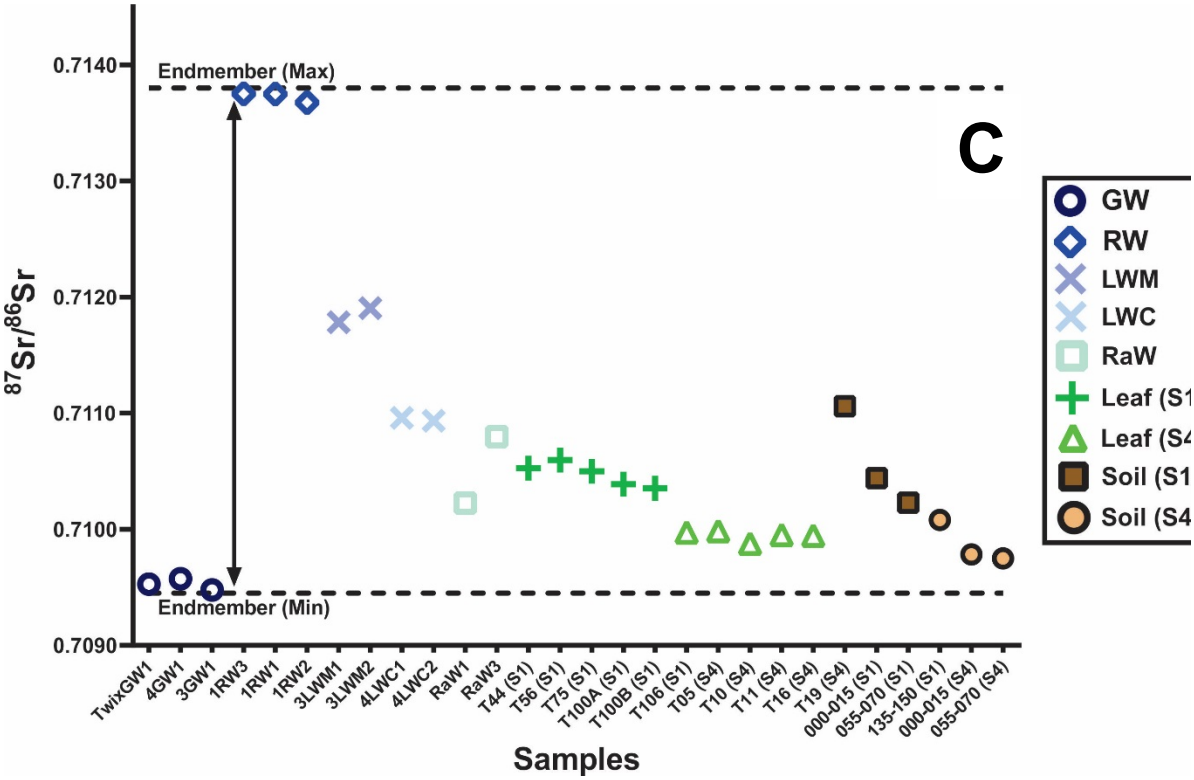


Figure 7 - Total samples plotted for $\delta^{18}O$ (A), δD (B) & $^{87}Sr/^{86}Sr$ (C). Dashed lines indicate the inferred minimum and maximum endmember values for surface and groundwater. GW = Groundwater. RW = River. LWM = Lake Merreti. LWC = Lake Clover. RaW = Rain. S1 = Site 1. S4 = Site 4. See **Table 1**

3.1.2. Soil Waters

Soil (S4) showed the least range with values -5.83 ‰ and -13.54 ‰ for $\delta^{18}O$, and -57.87 ‰ and -87.19 ‰ for δD (Figure 7a & 7b). Soil (S1) had a range of -10.70 ‰ and -16.08 ‰ for $\delta^{18}O$, and -61.78 ‰ and -105.35 ‰ for δD. Soil water, overall, did not differ greatly in mean values with -13.88 ‰ (S1) and -10.42 ‰ (S4) for $\delta^{18}O$, and -79.07 ‰ (S1) and -76.14 ‰ (S4) for δD. Note, samples that fell outside the value range were omitted from subsequent analysis and discussion as they were suspected as outliers resulting from the analytical procedure.

3.1.3. Stem Waters

Stem water (S1) values ranged from 0.88 ‰ to -14.59 ‰ for $\delta^{18}O$, and -19.21 ‰ to -130.41 ‰ for δD, while Stem water (S4) ranged from -2.66 ‰ and -6.18 ‰ for $\delta^{18}O$, and -28.87 ‰ and -87.36 ‰ for δD (Figure 7a & 7b). Stem water showed average values of -4.74 ‰ (S1) and -4.27 ‰ for (S4) for $\delta^{18}O$, and -48.3537 ‰ (S1) and -62.3496 (S4) for δD. Note, samples that fell outside the endmember range were ultimately omitted from subsequent analysis and discussion as they were suspected as outliers resulting from the analytical procedure.

3.1.4. $\delta^{18}O$ vs. δD Relationship

Water source values, characterizing a Local Evaporation Line (LEL, $\delta D = 5.5037\delta^{18}O + 7.2694$), from RaW to LW, increasingly deviated from the Global Meteoric Water Line (GMWL, $\delta D = 8^{18}O + 10$) (Figure 8). GNIR (Global Network of Isotopes in Rivers) values (n = 3) showed strong similarity to the determined LEL with only slight variation between both trend lines. Stem water values appeared to sit between RaW and GW for Stem (S4), and between GW and RW for Stem (S1).

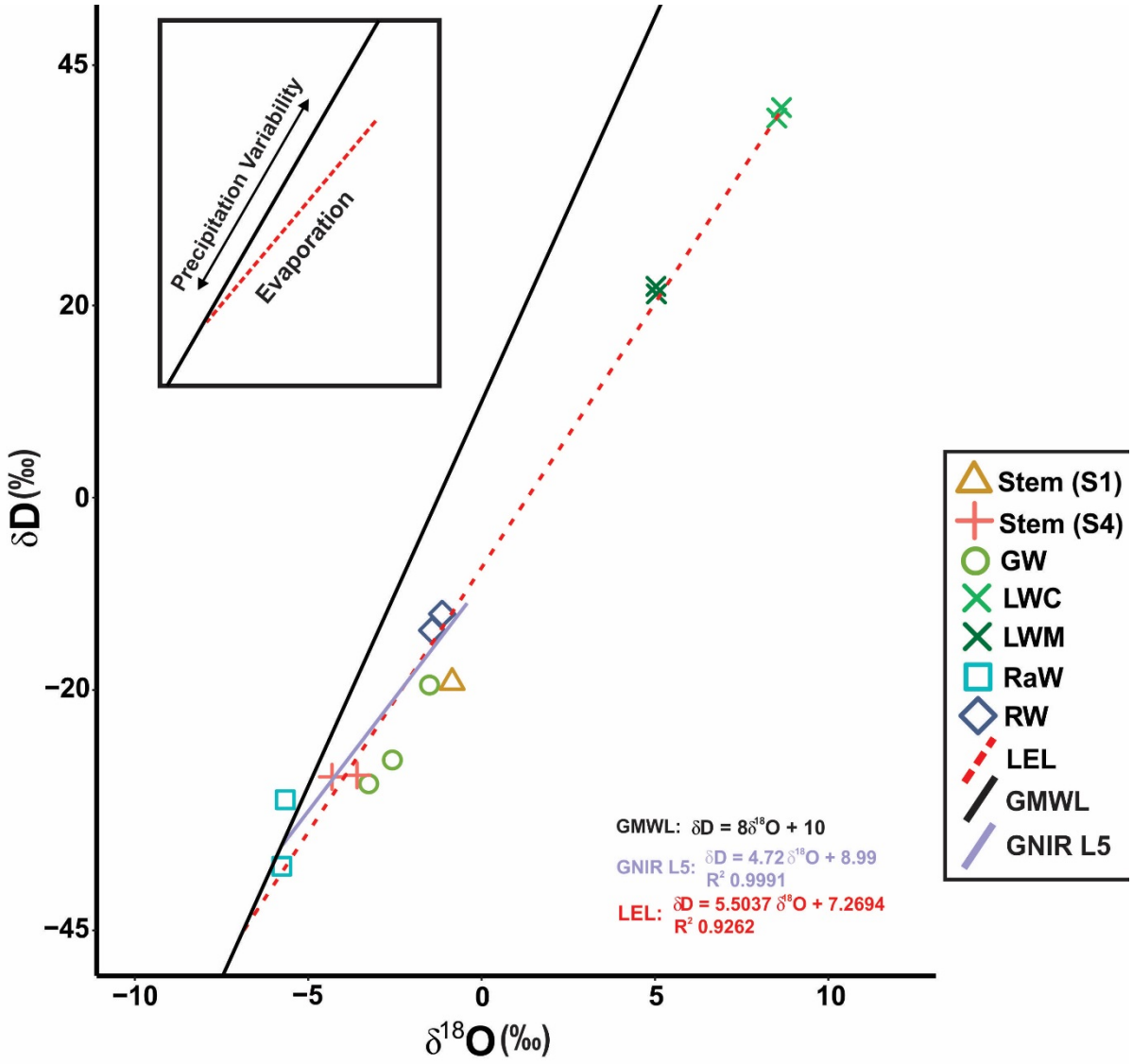


Figure 8 - $\delta^{18}O$ & δD surface and groundwater and stem data compared against Global Meteoric Water Line (GMWL - $\delta D = 8.0\delta$ 18O + 10‰) and Global Network of Isotopes in Rivers – Murray Lock 5 (GNIR L5) (n = 3) trend lines. LEL = Local Evaporation Line. GW = Groundwater. LWC = Lake Clover. LWM = Lake Merreti. RW = River. RaW = Rainwater. S1 = Site 1. S4 = Site 4. See **Table 1**

3.2. $^{87}Sr/^{86}Sr$

3.2.1. Surface and Groundwater

$^{87}Sr/^{86}Sr$ values for RW and GW ranged from 0.71374 for RW, and 0.7094 for GW (Figure 7c). LWM values fell between RW and GW with an average value of 0.7118. LWC

and RaW, on average, appeared closest to each other with values of 0.710947 for LWC, and 0.710512 for RaW. Both LWC and RaW values sat closest to GW (0.709526).

3.2.3. Soil Strontium

Bioavailable $^{87}Sr/^{86}Sr$ in soils, between sites, showed obvious differences with average $^{87}Sr/^{86}Sr$ values of 0.710577 for Soil (S1) and 0.709872 for Soil (S4) (Figure 7c). Soil (S1) showed a larger given range from 0.711059 to 0.710229 while Soil (S4) had a range of 0.710081 to 0.70975. The average value of Soil (S1), 0.710577, showed a relatively close relationship to RaW (0.710512). In contrast, average values of Soil (S4), 0.709872, showed a close relationship to GW (0.7094). Additionally, both sites exhibited a trend of decreasing $^{87}Sr/^{86}Sr$ with depth, ranging from 0.711059 (0 - 15 cm) to 0.710229 (135 - 15 cm) for Site 1, and a depth range of 0.710081 (0 - 15 cm) to 0.709750 (135 - 150 cm) for Site 4 (Figure 9).

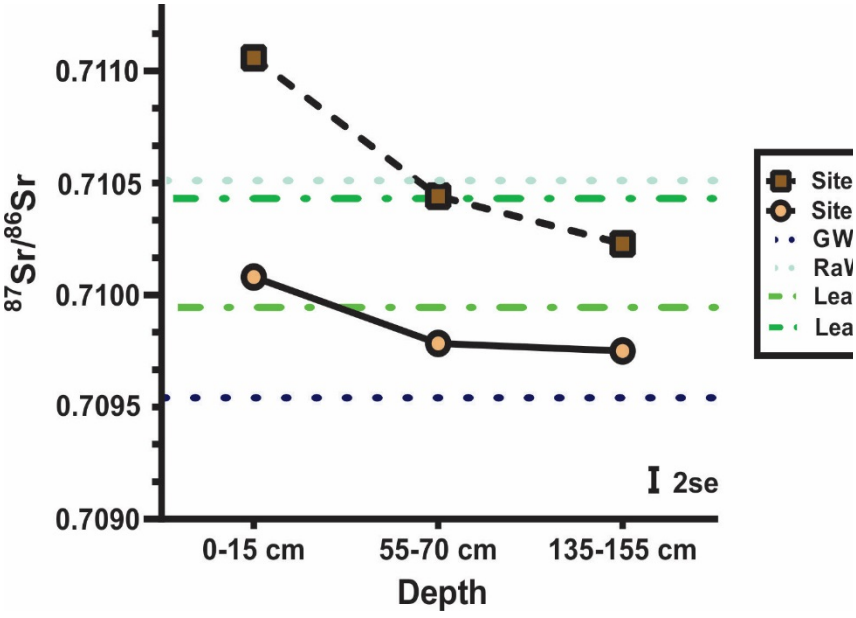


Figure 9 - $^{87}Sr/^{86}Sr$ & depth relationship for soil profiles from Site 1 and Site 4. Values categorized into 15 cm intervals. Dashed lines represent average leaf $^{87}Sr/^{86}Sr$ values, and dotted lines represent average GW (groundwater) and RaW (rainwater) values taken from study site. See Table 1. 2se = two-standard errors. See **Table 1**

3.2.2. Leaf Strontium

$^{87}Sr/^{86}Sr$ values for Leaf (S1) ranged from 0.710596 to 0.710352 while Leaf (S4) values ranged from 0.709986 to 0.709877. Leaf (S1) and Leaf (S4) had $^{87}Sr/^{86}Sr$ averages of 0.710458 and 0.709947, respectively (Figure 7c). Average Soil (S1) values showed a close relationship to Leaf (S1), 0.710458, and Soil (S4) showed a close relationship to Leaf (S4), 0.709947.

3.3. Multi-isotope Comparison Plots

3.3.1. $^{87}Sr/^{86}Sr$-Oxygen

Water sources showed consistent differences and separation for both $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ (Figure 10). Both RaW samples had similar $\delta^{18}O$ values but differed in $^{87}Sr/^{86}Sr$. LWM, LWC and GW all clustered together with only small differences across both $\delta^{18}O$ and $^{87}Sr/^{86}Sr$. Tree (S1) and Tree (S4) showed relatively consistent values for $^{87}Sr/^{86}Sr$ and notable differences due to enriched $\delta^{18}O$ values of Tree (S1). Overall, tree values were closest to RaW and GW. Specifically, Tree (S1) was relatively closer to RaW while Tree (S4) was closer to GW.

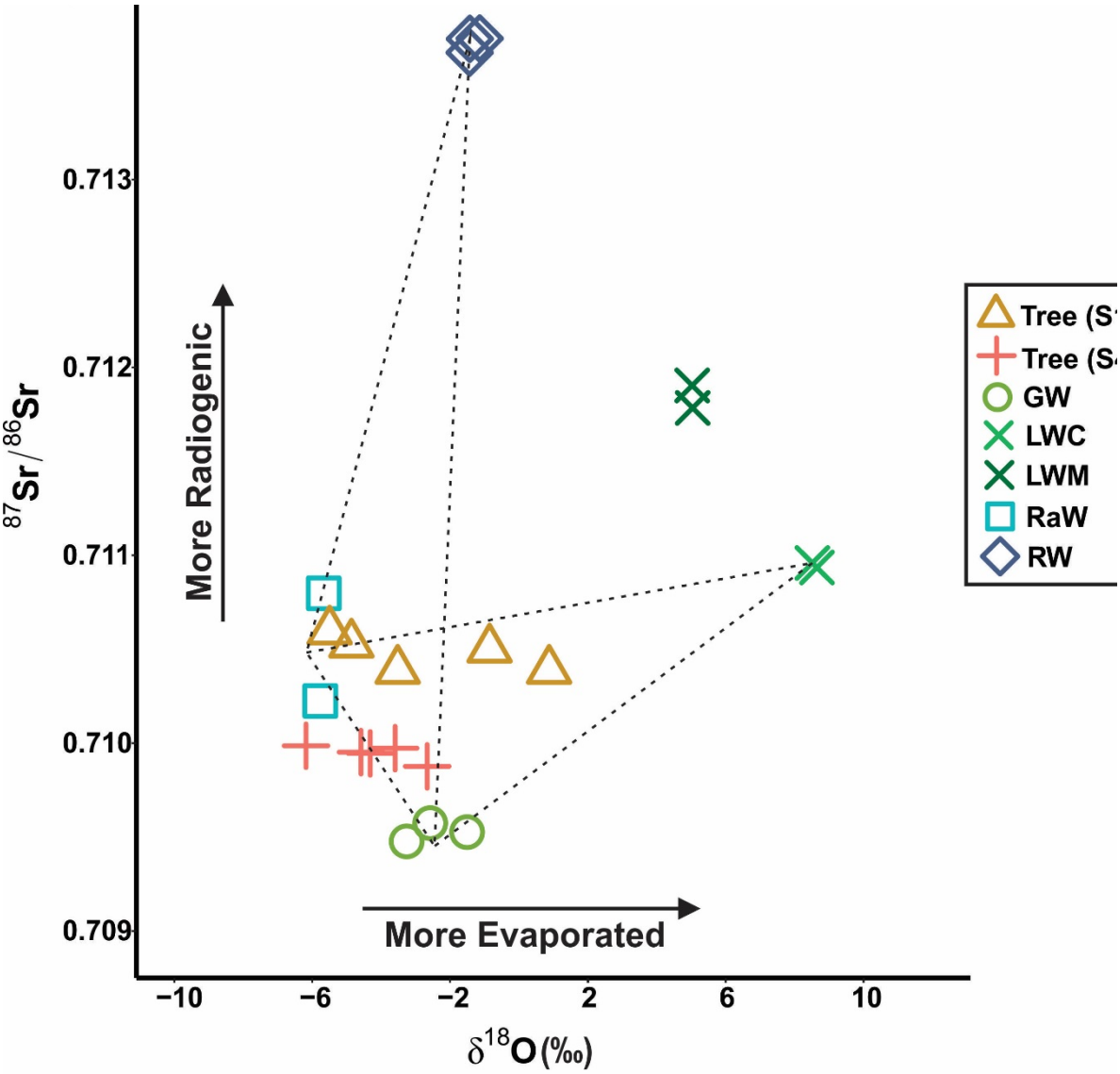


Figure 10 - $\delta^{18}O$ & $^{87}Sr/^{86}Sr$ plot for tree (stem/leaf) and surface and groundwater samples. Dashed lines express hypothetical mixing lines between water source averages. GW = Groundwater. LWC = Lake Clover. LWM = Lake Merreti. RW = River. RaW = Rainwater. S1 = Site 1. S4 = Site 4. Tree = Stem/Leaf data. See **Table 1.**

Table 1 - $\delta^{18}O$, δD, $^{87}Sr/^{86}Sr$, depth and salinity data for samples collected from Site 1 & Site 4. Water sources: GW = Groundwater. LWC = Lake Clover. LWM = Lake Merreti. RW = River. RaW = Rainwater. GNIR = Global Network of Isotopes in Rivers – Lock 5. S1 = Site 1. S4 = Site 4. 2se = two-standard errors.

Sample ID	Type	Site	$\delta^{18}O$	δD	$^{87}Sr/^{86}Sr$	Depth (cm)	Salinity (PSU)	2se	Notes
TwixGW1	GW	3 & 4	-1.50	-19.48	.709527		36.07	.000003	
4GW1	GW	4	-2.57	-27.28	.709574		39.38	.000003	
3GW1	GW	3	-3.26	-29.75	.709477		48.09	.000003	
3LWM1	LWM	3	5.04	21.21	.711784		0.28	.000003	
4LWC1	LWC	4	8.51	39.50	.710959		0.73	.000003	
4LWC2	LWC	4	8.65	40.58	.710935		0.74	.000003	
3LWM2	LWM	3	5.03	21.99	.711904		0.29	.000005	
1RW3	RW	1	-1.14	-12.08	.713751		0.13	.000003	Big4 Renmatk Caravan Park (location)
1RW1	RW	1	-1.42	-13.75	.713749		0.13	.000003	
1RW2	RW	1	-1.43	-13.79	.713676		0.13	.000004	
RaW1	RaW	Calperum Station	-5.76	-38.33	.710225		0-0.1	.000002	17/5/2018 – 17/6/2018
RaW3	RaW	Calperum Station	-5.66	-31.42	.710798		0-0.1	.000004	17/7/2018 – 17/8/2018
GNIR L5	GNIR	Lock 5	-2.54	-21.2					Murray River - IAEA
GNIR L5	GNIR	Lock 5	-1.73	-17.00					Murray River - IAEA
GNIR L5	GNIR	Lock 5	-5.41	-34.50					Murray River - IAEA
T05	Stem	4	-3.59	-28.87	.709973			.000003	
T11	Stem	4	-6.18	-56.30	.709986			.000003	
T10	Stem	4	-2.66	-87.36	.709877			.000003	
T19	Stem	4	-4.31	-29.04	.709946			.000003	
T16	Stem	4	-4.58	-40.20	.709952			.000004	Alcohol contaminated
T56	Stem	1	-5.49	-43.09	.710596			.000004	Alcohol contaminated
T75	Stem	1	-0.85	-19.21	.710500			.000003	
T100A	Stem	1	-3.52	-62.40	.710388			.000003	
T100B	Stem	1	0.88	-24.90	.710388			.000003	Alcohol contaminated
T106	Stem	1	-14.59	-130.41	.710352			.000003	Alcohol contaminated
T44	Stem	1	-4.86	-94.09	.710527			.000003	Alcohol contaminated
S1000-015	Soil	1	-10.71	-61.78	.711059	0-15		.000003	Alcohol contaminated
S1055-070	Soil	1	-16.08	-105.35	.710441	55-70		.000003	
S1135-150	Soil	1	-14.84	-70.10	.710229	135-150		.000005	
S4000-015	Soil	4	-5.83	-57.87	.710081	0-15		.000004	
S4055-070	Soil	4	-13.54	-87.19	.709784	55-70		.000003	
S4135-150	Soil	4	-11.89	-83.38	.709750	135-150		.000003	

3.4. Mixing Models

Both groundwater (GW) and rainwater (RaW) was selected as endmembers for both models based on the assumption that they did not vary between sites. However, the third endmember was varied and chosen based on spatial distance e.g. lake (LW) for Site 4 and river (RW) for Site 1 (Figure 11 & 12). Stem (S1) values for $\delta^{18}O$ data showed varying percentages ranging between 35-95% for RaW, 5-10% for RW, and 0-60% for GW**.** Leaf (S1) values for $^{87}Sr/^{86}Sr$ ranged from 74-84% for RaW, 3-10% for RW, and 13-20% for GW. Soil (S1) values for $^{87}Sr/^{86}Sr$ ranged from 69-83% for RaW, 0-18% for RW, and 0-30% for GW. Stem (S4) values for $\delta^{18}O$ ranged from 10-65% for RaW, 0% for LWC, and 30-90% for GW**.** Leaf (S4) values for $^{87}Sr/^{86}Sr$ ranged from 31-38% for RaW, 5-10% for LWC, and 60-70% for GW. Soil (S4) values for $^{87}Sr/^{86}Sr$ ranged from 0-25% for RaW, 0-40% for LWC, and 60-78% for GW. For Site 1, average endmember percentages for Soil ($^{87}Sr/^{86}Sr$) were 77% for RaW, 16% for GW, and 7% for RW. Leaf ($^{87}Sr/^{86}Sr$) average percentages were 77% for RaW, 17% for GW, and 6% for RW. Stem ($\delta^{18}O$) average percentages were 67% for RaW, 26% for GW, and 7% for RW. Finally, for Site 4, average endmember percentages for Soil ($^{87}Sr/^{86}Sr$) percentages were 16% for RaW, 70% for GW, and 14% for LWC. Leaf ($^{87}Sr/^{86}Sr$) average percentages were 32% for RaW, 62% for GW, and 6% for LWC. Stem ($\delta^{18}O$) average percentages 42% for RaW, 58% for GW, and 0% for LWC. Note, only $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ isotope data was used as mixing model inputs due to δD samples having high variability.

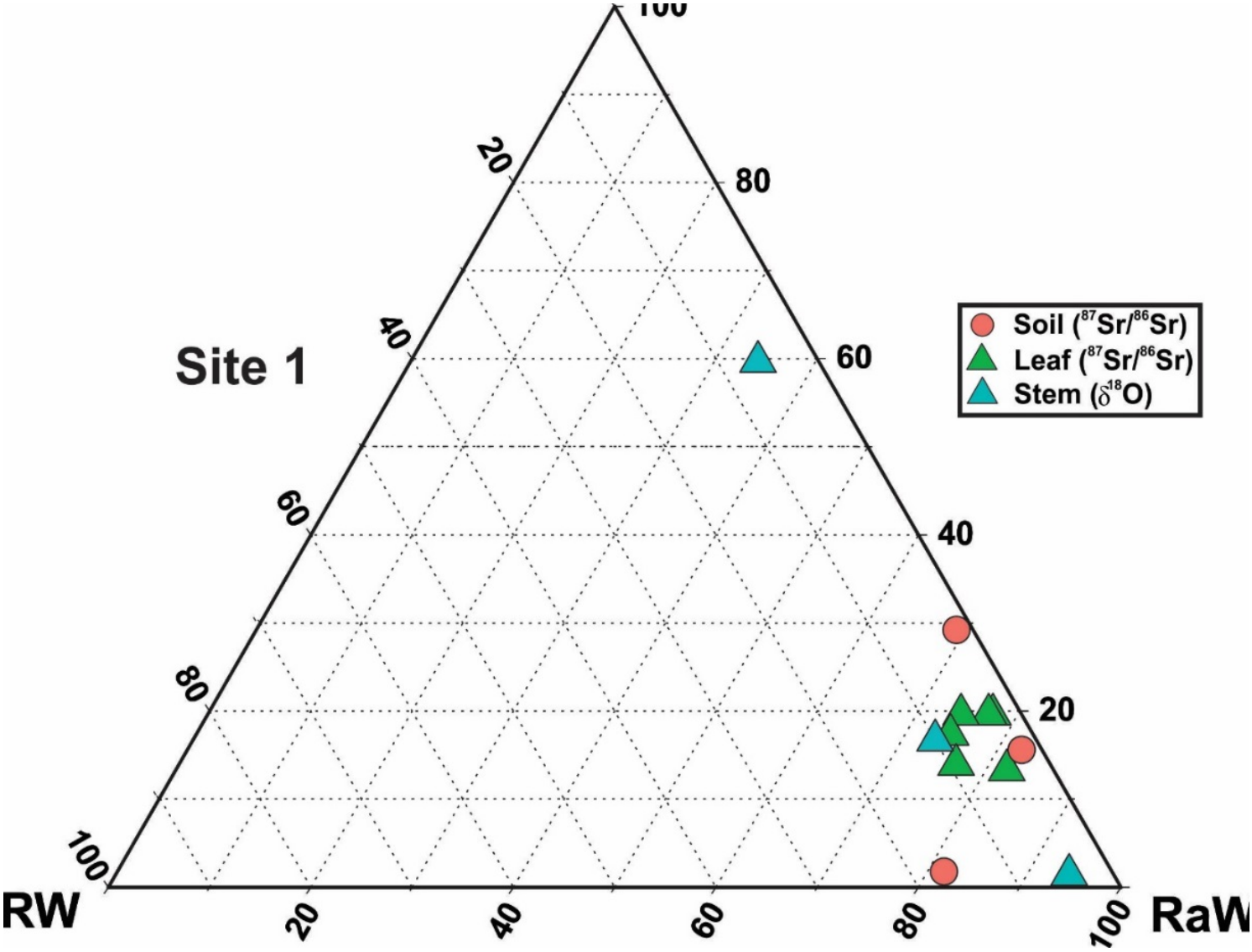


Figure 11 - Ternary plots showing the proportional water source inputs (%) for soil and organic material (leaf & stem) sampled at Site 1 for $\delta^{18}O$ and $^{87}Sr/^{86}Sr$. Samples were included based on inferred water source range (see Figure 7). GW = Groundwater. RW = River water. RaW = Rainwater. See **Table 2.**

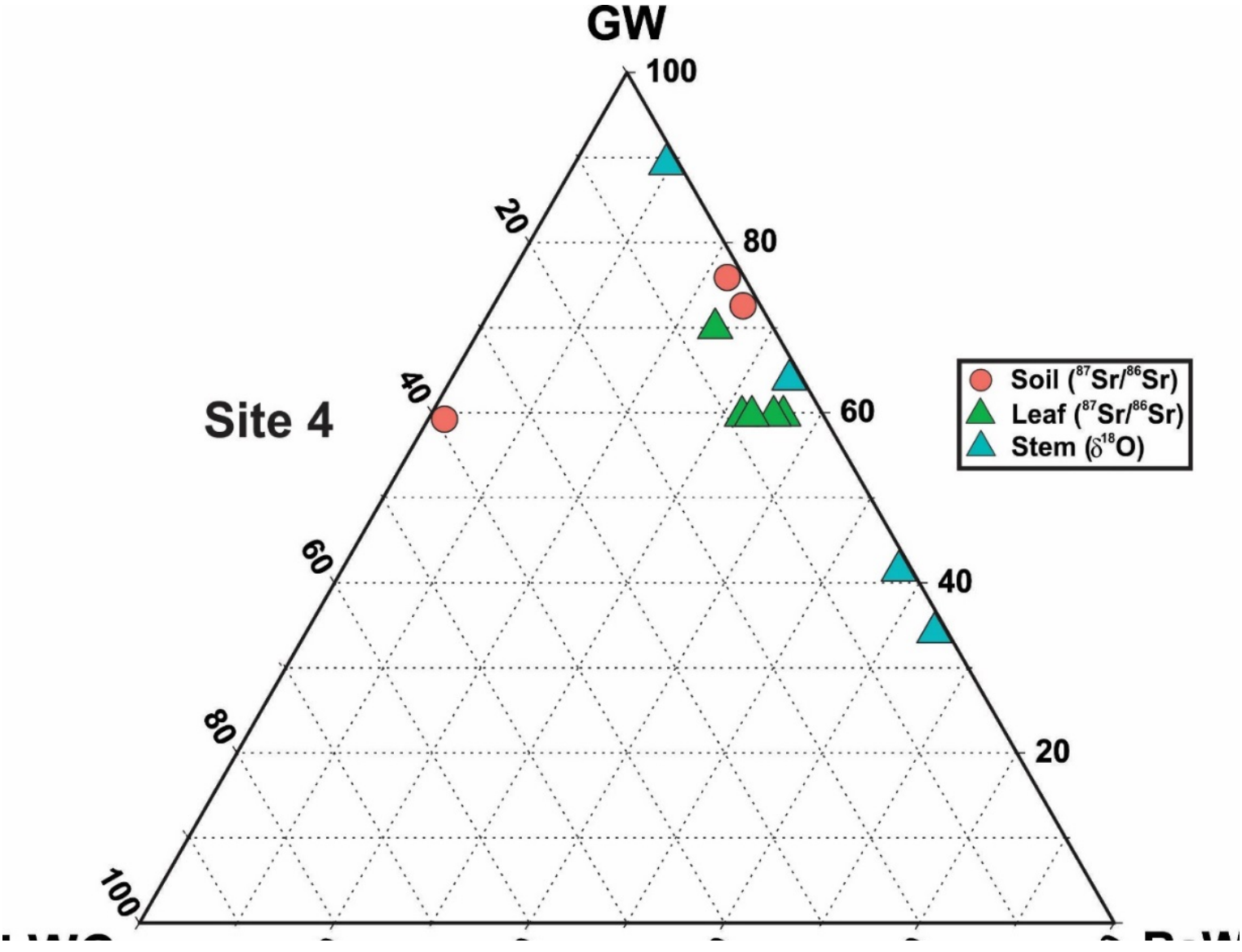


Figure 12 - Ternary plots showing the proportional water source inputs (%) for soil and organic material (leaf & stem) sampled at Site 4 for $\delta^{18}O$ and $^{87}Sr/^{86}Sr$. Samples were included based on inferred water source range (see Figure 7). GW = Groundwater. RW = River water. RaW = Rainwater. See **Table 2.**

Table 2 - Used endmembers and determined isotope fractions ($^{87}Sr/^{86}Sr$ & $\delta^{18}O$) for linear mixing models. Px = Parameter. Fx = Fraction. M = Mixture/Total value. Equation: $\delta m = \delta afa + \delta bfb + \delta cfc$ and $1 = fa + fb + fc$.

Sample ID	Tracer	Site	Pa	Pb	Pc	Fa	Fb	Fc	M
			Model						
S1000-015	$\delta^{18}O$	1	RW	GW	RaW	n/a	n/a	n/a	n/a
S1055-070	$\delta^{18}O$	1	RW	GW	RaW	n/a	n/a	n/a	n/a
S1135-150	$\delta^{18}O$	1	RW	GW	RaW	n/a	n/a	n/a	n/a
S4000-015	$\delta^{18}O$	4	LWC	GW	RaW	n/a	n/a	n/a	n/a
S4055-070	$\delta^{18}O$	4	LWC	GW	RaW	n/a	n/a	n/a	n/a
S4135-150	$\delta^{18}O$	4	LWC	GW	RaW	n/a	n/a	n/a	n/a
T106	$\delta^{18}O$	1	RW	GW	RaW	n/a	n/a	n/a	n/a
T100A	$\delta^{18}O$	1	RW	GW	RaW	0.05345	0.6	0.34655	-3.518169
T100B	$\delta^{18}O$	1	RW	GW	RaW	n/a	n/a	n/a	n/a
T75	$\delta^{18}O$	1	RW	GW	RaW	n/a	n/a	n/a	n/a
T56	$\delta^{18}O$	1	RW	GW	RaW	0.050426	0.01	0.9496748	-5.49283
T44	$\delta^{18}O$	1	RW	GW	RaW	0.1	0.1611	0.7389	-4.859922
T19	$\delta^{18}O$	4	LWC	GW	RaW	0.01	0.42798	0.57202	-4.314575
T16	$\delta^{18}O$	4	LWC	GW	RaW	0.01	0.34955	0.65255	-4.582901
T11	$\delta^{18}O$	4	LWC	GW	RaW	0.0101	0.9	0.0899	-2.662200
T10	$\delta^{18}O$	4	LWC	GW	RaW	n/a	n/a	n/a	n/a
T05	$\delta^{18}O$	4	LWC	GW	RaW	0.01	0.649	0.351	-3.5929144
S1000-015	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.1705	0.1	0.8295	0.711059
S1055-070	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.0239	0.15	0.8261	0.710441
S1135-150	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.002099	0.3	0.697911	0.710229
S4000-015	$^{87}Sr/^{86}Sr$	4	LWC	GW	RaW	0.01	0.739	0.261	0.710081
S4055-070	$^{87}Sr/^{86}Sr$	4	LWC	GW	RaW	0.01	0.769	0.231	0.709784
S4135-150	$^{87}Sr/^{86}Sr$	4	LWC	GW	RaW	0.01	0.739	0.261	0.709750
T106	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.091	0.15	0.759	.710630
T100A	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.0227	0.2	0.7773	.710388
T100B	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.0227	0.2	0.7773	.710388
T75	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.05001	0.13	0.81999	.710500
T56	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.0797	0.18	0.7403	.710596
T44	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.0583	0.2	0.7417	.710527
T19	$^{87}Sr/^{86}Sr$	4	LWC	GW	RaW	0.0411	0.6	0.3589	.709946
T16	$^{87}Sr/^{86}Sr$	4	LWC	GW	RaW	0.0511	0.6	0.3489	.709952
T11	$^{87}Sr/^{86}Sr$	4	LWC	GW	RaW	0.0811	0.6	0.3189	.709986
T10	$^{87}Sr/^{86}Sr$	4	LWC	GW	RaW	0.0611	0.7	0.2389	.709877
T05	$^{87}Sr/^{86}Sr$	4	LWC	GW	RaW	0.0711	0.6	0.3289	.709973

4. DISCUSSION

4.1. Local Surface and Groundwater Hydrology

Surface water and groundwater, across the Calperum Floodplain, showed distinct isotopic signatures. Compared to RW, GW, and RaW, $\delta^{18}O$ and δD revealed that both LWM and LWC, along the Local Evaporation Line, were relatively more evaporated (Figure 8). Additionally, $^{87}Sr/^{86}Sr$ data demonstrated that LMC received most of its $^{87}Sr/^{86}Sr$ from RaW while LWM revealed a relative mix between RW and RaW (Figure 10). These findings are consistent and in confirmation with known local hydrology because LWC is descriptively an evaporative lake reliant on rainwater and LWM in the past was maintained via artificial connection to the River Murray (MDBA, 2010). $\delta^{18}O$ and δD values for RW appeared to show a validating association with historical (GNIR) river datasets (Figure 8)**.** Deviation of river water from the GMWL is common in arid systems (Gibson et al., 2002) and could explain why both datasets showed a varied but similar evaporation offset. It was expected that RaW and GW would show a strong similarity due to groundwater being commonly recharged by precipitation in semi-arid environments (Edmunds, 2001). However, based on $\delta^{18}O$ and δD values, it appeared that GW was not reflective of local precipitation, and subject to evaporation (Figure 8). Moreover, $^{87}Sr/^{86}Sr$ data also showed no similarity with precipitation and had a close similarity to seawater (IAPSO standard[5]), indicating that GW sourcing was not completely RaW derived (Figure 10). Possible explanations for the overall lack of similarity between GW and RaW could have been due to limited soil infiltration from precipitation or the incorporation of paleo-water (Hornberger, 1999). In terms of GW-RW, there appeared to be a notable overlap between $\delta^{18}O$, suggesting that RW was a recharge component of GW or vice versa (Figure 10). However, $^{87}Sr/^{86}Sr$ values indicated that both GW and RW were derived from similar sources (Figure 10). Previous work on the River

[5] See Appendix B

Murray floodplains has revealed that groundwater is recharged by lateral flow from nearby streams (Holland et al., 2006), however, since groundwater samples were not taken near RW the likelihood of recharge, in this case, is low, and does not explain the overlap in $\delta^{18}O$. Finally, RaW values, from the first and third month, varied in $^{87}Sr/^{86}Sr$ but not in $\delta^{18}O$ (Figure 10). A possible explanation for the difference in $^{87}Sr/^{86}Sr$ accuracy values could have been the inclusion of different atmospheric dust sources acro*s*s monthly precipitation events (Négrel, Guerrot & Millot, 2007). In conclusion, based on $^{87}Sr/^{86}Sr$, $\delta^{18}O$ and δD data, it appeared that sampled water sources were clearly isotopically independent from one another, consistently similar across sampled replicates, and in agreement with known local hydrology.

4.2. Comparing Soil, Water and Black Box Tree Interactions Using $\delta^{18}O$ & $^{87}Sr/^{86}Sr$

On average, $\delta^{18}O$ showed no relationship between sampled soil and local water sources (Figure 7a). This could be attributed to cryogenic distillation procedure which has been subject to lengthy discussion regarding its validity and accuracy regarding water extraction (Araguás-Araguás et al, 1995; Goebel & Lascano, 2012; Koeniger et al., 2011; Millar et al., 2018; Orlowski et al., 2018; Orlowski, Breuer & McDonnell, 2016; Orlowski, Frede, Brüggemann & Breuer, 2013; Peters & Yakir, 2008; West, Patrickson & Ehleringer, 2006). However, $\delta^{18}O$ still revealed that Site 1 trees exhibited use of evaporated rainwater (Figure 10) which suggests that these trees were mainly sourcing their water from the upper surface soils (Hu et al., 2008). Additionally, $\delta^{18}O$ showed that Site 4 trees appeared to be reliant on a varied mix of GW and RaW (Figure 10). In contrast, $^{87}Sr/^{86}Sr$ revealed that soils and leaf material were predominately associated with RaW and GW for both sites (Figure 7c). Additionally, $^{87}Sr/^{86}Sr$ showed a close overlap between leaf and soil strontium samples (Figure 7c). $^{87}Sr/^{86}Sr$ depth profiles appeared to suggest that that RW was prominent at 55-70 cm depths for Site 1 (Figure 9). However, there is a risk in inferring relationships because

soils could be contaminated by decomposing litter fall or organics (Poszwa et al., 2009). Both $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ data confirms previous findings that found Black Box trees are naturally reliant on groundwater and/or rainwater-derived soils during inter-flooding periods (Jolly, & Walker, 1996). In conclusion, inferred relationships between soil, water and Black Box trees drastically varied between $^{87}Sr/^{86}Sr$ and $\delta^{18}O$. Comparatively, $^{87}Sr/^{86}Sr$, on average, appeared to be a more conservative tracer due its insensitivity to fractionation (Flockhart et al., 2015), and because of issues regarding cryogenic distillation. However, regardless of this, there are still limitations in using $^{87}Sr/^{86}Sr$ to analyse soils because $^{87}Sr/^{86}Sr$ values may still be influenced by decomposed organic material or water-sediment interactions.

4.3. Black Box Tree Water Use Patterns

Black Box tree water source preferences varied distinctly between Site 1 and Site 4 (Figure 11 & 12). $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ data showed that Site 1 trees had a greater uptake preference for RaW (Soil 77%, Leaf 77% & Stem 67%) while Site 4 trees favoured more GW (Soil 70%, Leaf 62% & Stem 58%). RaW preference was also prominent in Site 4 trees (Soil 16%, Leaf 32% & Stem 42%) while Site 1 trees consumed overall less GW (Soil 16%, Leaf 17% & Stem 26 %) and only preferred RaW. One explanation for the difference in water source preferences could be due to groundwater accessibility and associated factors regarding salinity, soil stratigraphy and depth to groundwater, and flooding frequency. Depth to groundwater could explain use preference since historical drill records (WaterConnect, 2018; 1991-2009) appeared to show that Site 1 groundwater (17 m) when compared to Site 4 (5 m) was much deeper. However, this could be irrelevant since eucalyptus spp. have the potential of growing root systems up to 40 m (Canadell et al., 1996). Groundwater salinity at

Site 4 (48 PSU[6]) exceeded known limits (25.5 PSU[7]; DWLBC, 2004), yet salinity at Site 1 (2.2 PSU[8]) did not (WaterConnect, 2018; 1998). Considerable lower salinity and groundwater depth at Site 1 could most likely be a product of nearby river infiltration. At Site 4, higher groundwater tables and salinity is consistent with both an observed presence of epicormic growth – a typical indicator of stress (Meier, Saunders & Michler, 2012) – and lower tree abundance. Relative tree health and high groundwater tables at Site 4, therefore, could explain why these Black Box trees seemed to be using groundwater. Smaller flooding extents, closer to the River Murray (Site 1), are more frequent than larger ones which are needed to extend to the northern floodplain regions (Site 4) (MDBA, 2012). Tree material may not have reflected floodwater use but historically it could be assumed that Site 1 trees survive on rainwater between flooding periods, and could explain their lack of reliance on deeper groundwater. Additionally, assuming rainfall distribution is spatially homogenous, and soil stratigraphy is asymmetrical between sites, it could be likely that the trees at Site 1 only seemed to prefer RaW because they are unable to penetrate Site 1 upper clay capping (2-5 m), therefore causing the trees to be restricted to only rainfall and, perhaps, infrequent flooding (Holland, Overton & Walker, 2004; WaterConnect, 2018).

In conclusion, due to (i) the lack of accurate ecological (height, size, root-zone depth etc.) and hydro-stratigraphic data (soil composition, porosity etc.), and (ii) absence of long term monitoring of groundwater depth and precipitation, drawing conclusions about why certain Black Box tree populations prefer one source over another is speculative. However, based on what is known, groundwater and soil water accessibility appear the most likely explanations for why Black Box trees had a greater preference for RaW at Site 1 and GW at

[6] See Appendix B
[7] Converted from EC (mS/cm)
[8] Converted from EC (uS/cm)

Site 4.

4.4. Management Implications

Based on the above findings it appeared that both Black Box tree populations prefer to use either RaW and/or GW, regardless of nearby lakes or streams. This has significant water management implications since access to groundwater is heavily dependent on groundwater depth and salinity which is, in turn, influenced by degree of infiltration of rainfall and flooding inundation (Agriculture Victoria, 2018; McEwan, Jolly & Holland, 2006). Additionally, it is known that Black Box trees subsist if flooding or rainfall events occur every 8 to 10 years (George, 2004). Also, stable floodplain ecosystems require the establishment of new seedlings which is ultimately dependent on frequent short term flooding events (Rogers & Ralph, 2011; Treloar, 1959). However, due to the impact of climate change, upstream irrigation practices and agriculture (Banks & Docker, 2014; Pachauri & Meyer, 2015), the probability of natural flows reaching outer Black Box tree populations is negligible. Alternatively, River Murray connected water pumps may be a possible solution by helping to target outer floodplain regions and providing water stress relief to declining Black Box populations (George, Walker & Lewis, 2005; Moxham, Duncan & Moloney, 2017). Unless considerable changes are made, regarding artificial flooding and site allocated water, northern Black Box tree population health and establishment may continue to decline radically over the next few decades.

4.5. Future Studies

In the future, a number of modifications and additions to the chosen samples and methods could be made. For instance, stem sampling could specifically focus on the collection of older and woodier stems, rather than bulk sampling. Evidence suggests that

water from older and younger stems can vary isotopically (Ellsworth, & Williams, 2007) due to xylem-phloem exchange in younger stem growth (Martín-Gómez, Serrano & Ferrio, 2016). Isotopic analysis of sampled tree-ring cores could be sampled because they have the potential to reduce uncertainty surrounding current and historical water use (Epstein et al., 1990). Atmospheric dust has been known to make up 80% of incorporated bioavailable strontium in plants (English et al., 2001) and could be used as an additional endmember in calculated mixing models and used to account for $^{87}Sr/^{86}Sr$ variations in precipitation**.** Deeper sampled soil profiles could provide greater certainty regarding soil water use depth of soil water use (Dawson et al., 2002; George et al., 2005; Thorburn & Ehleringer, 1995). Additionally, soil and predawn water potential could be sampled to determine water uptake depth because plants are more likely to extract water from soil zones that are more or less equal to plant predawn water potential (Holland et al., 2006; Zubrinich et al., 2000). Long term measurements of depth-to-groundwater and precipitation could also help constrain water accessibility and help explain why trees prefer one source over another. Additionally, cryogenic distillation procedures were unreliable which suggests that methods need to be validated via controlled experiments using spiked standards, and varying pressure, temperature, and extraction time parameters (Orlowski, Breuer & McDonnell, 2016). In summary, future research could benefit from the sampling of older stems, tree-ring cores, atmospheric dust, deeper soil profiles, soil-water potential and groundwater depth, to help better account for compounding environmental variables, and improve resolution of relationships between soil, water and Black Box trees. Finally, future research should focus on validating cryogenic distillation so that stem and soil water data can be confidently analysed.

5. CONCLUSION

In conclusion, $^{87}Sr/^{86}Sr$, $\delta^{18}O$ and δD isotopes confirmed that local water sources were isotopically distinct and consistent with known local hydrology. Additionally, $^{87}Sr/^{86}Sr$ revealed that site specific water, soils and Black Box trees showed a close relationship with one another, and was, overall, a more reliable tracer than $\delta^{18}O$. Linear mixing models confirmed initial expectations and found that Black Box trees favoured groundwater and rainwater regardless of nearby lakes and streams. However, proportional water use preferences varied between Site 1 and Site 4 with the former being predominantly reliant on rainwater. Site differences were speculated to be caused by variation of groundwater accessibility due to soil stratigraphy and distance from the River Murray. These findings were concluded to have significant management implications regarding the allocation of water flows to Black Box tree populations on the outer floodplain regions. Overall, although validated against previous findings, there was still unresolved uncertainties regarding water use patterns due to issues with cryogenic distillation methods, limited soil stratigraphic and ecological data. In the future, research should focus on sampling deeper soil profiles, measuring water-soil potential, validating cryogenic distillation methodologies, analysing tree-ring cores, and tracking groundwater depth and rain variability, long term, to reduce uncertainty regarding the water use differences between Black Box tree populations.

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Appendix A: EXTENDED METHODLOGY

A.1. Sampling

A.1.1 Water

Water samples were stored in 250 mL and 150 mL polyethylene capped bottles. Water samples collected for Sr analysis were stored in pre-acid washed 250 mL bottles while samples collected for δH and δO analysis were stored in 150 mL HDPE bottles. Care was made so that no contaminated matter entered the Sr samples bottles and no bubbles were present in the δH and δO sample bottles during and post-sampling. Sample bottles were either left in a cool esky or kept out of the sun in the car. Two replicates for Lake Clover and Lake Merreti and three replicates from the River Murray were collected. Replicates for both the river and lake samples were taken at least >1 km apart. River sample 1 (1RW1) and 2 (2RW1) were both collected closest to Site 1. However, river sample 3 (3RW1) was taken ~25 km south at Big4 Renmark Caravan park. Groundwater was taken from pre-drilled bores scattered across the floodplain and closest to Site and Site 4. 4GW1 was taken from borehole BRS 17 which was closest to Lake Clover. 3GW1 was take from borehole BRS 6 which, in turn, was closest to Lake Merreti. Sample Twix3-4GW1 was taken from a bore hole in between 4GW1 and 3GW1, but no defined spatial location was recorded at the time of collection. Sampling at borehole MU OW 18 was inaccessible due to floodplain conditions and borehole quality. Therefore, groundwater data for Site 1 was not obtained. Procedure for groundwater collection involved using a small DIY pump and car battery to extract samples bores. During extraction the top layer of water was exhumed first, for a 2 minute duration, to reduce potential contamination or fractionation. Rainwater water was collected over the course of +3 months at Calperum station using two rain collectors. Collectors used were made up of a plastic bracket(s), plastic funnel(s), a 1 litre polyethylene for Sr and a 1 litre glass schott for δH/δO. Plastic centre structures were constructed to prevent sediment and contamination in flux**.** Water was collected over the months of April, June, July and August. Rainwater samples were finally obtained and put in cool storage on the 26th of August, ready for analysis.

A.2 Analysis

A.2.1 Strontium

A.2.1.1 Laboratory Materials and Acids

All chemistry preparation and Strontium extraction was carried out in a Class 1000 over-pressured ultraclean laboratory in the basement of the Mawson Building, University of Adelaide. AR grade acids for both cleaning and protocol completion were purified prior to use by Savillex DST-1000 sub-boiling distillation system. Deionized (DI) water was filtered using Ultrapure Milli-Q H2O at a resistivity of 18.2 MΩ cm-1 and used throughout all chemistry processes and protocols. Savillex PFA Teflon vials and centrifuge tubes were all pre-cleaned before samples came in contact. Teflon vials were cleaned using recycled 6 M HCl, 6 M HNO3, 6 M HCl and DI Milli-Q H2O across a combined total of 6-7 days. The vials were then rinsed, dried and stoed ready for sample collection.

A.2.1.2 Soil and Ammonium Acetate

An ammonium acetate protocol, based on a modified version from Reeuwijk (2002), was used in the liberation of Sr cations from soil exchangeable sites. Six soil samples and one blank were weighed out using a digital analytical balance to 2.0 g and placed in 50 ml centrifuge tubes**.** 20 ml of 1 M ammonium acetate was then added and 15.7 g of Ammonium acetate was diluted with 200 ml of DI water. Mixed samples were then placed in a rotor shaker for 2 hours and transferred to a centrifuge for 30 mins at 4,000 g (Centrifuge 5804 eppendorf) to separate solids from liquids. The supernatant liquid was then transferred carefully into Teflon vials and put aside for evaporation on a hotplate, and ready for analysis.

A.2.1.3 Leaf Ashing

Before use eleven small ceramic crucibles were submerged and acid washed with HCl overnight. 3-5 leaves of varying size, from 10 different sampled trees, were then chosen to be ashed. Care was taken to pick leaves that did not have nodules or diseased spots to prevent potential Sr contamination. These leaves were then washed with DI water and dried overnight before being broken up and placed in an oven at 1050 C for 24 hours. The ashed material was then placed into clean Teflon vials and left aside for analysis. It is to be noted that some samples were ashed multiple times due to the insufficient amount of leaf material available. Other samples (e.g. T106) did not go to completion (high organics) and affected Sr analysis, requiring repeated preparation and analysis. Blanks accompanied the entire process to account and correct for contamination from the oven and above procedures.

A.2.1.4 Water Evaporation

River, rain, lake and groundwater samples were transferred into Teflon vials and evaporated overnight on a hotplate at 150 C. Evaporation was used to precipitate out dissolved materials for analysis. Most liquid samples only required <20 ml of water, however, additional volume (200 ml) was required for rainwater which does not have naturally high concentration levels of Sr. Rain water was left to evaporate over 3-4 days to

acquire enough precipitates. Evaporation precipitates were then left aside in their capped vials and stored ready for Sr Micro Bio-Spin column procedure.

A.2.1.5 Sr Micro Bio-Spin column procedure

Columns were first removed from 1 M HNO3 storage container using blue PP forceps. These columns were rinsed 3 times with DI water and placed into plastic racks. While avoiding bubbles the columns were then loaded, using a 1 ml pipette, with 200 uL of Sr Resin SPS, filling up ⅓. Before final Sr extraction took place a resin cleaning procedure was followed. This procedure involved washing the resin and columns cyclically with 3 mL 8M HNO3 sd and 3 mL of DI H2O, twice. Columns were then equilibrated using 3 ml of HNO3 sd before sample loading. Sample precipitates were then dissolved in 1 mL of 8M HNO3 sd and carefully loaded into the columns. Resin and columns were subsequently washed with 5 mL of 8M HNO3 and freshly cleaned Teflon vials were then placed underneath the racked columns. Sr was proceeded to be collected via rinsing the column resin six times using 0.05M HNO3 sd. One drop of 0.1M H3PO4 was then added to each Teflon vial and left on a hotplate to be dried at 140 C until all liquid had evaporated. After evaporation, samples that had any residual black organics were then subjected to a drop of 15M HNO3 and left to dry on a hotplate overnight at 140 C. Once all black organics had been dissolved and a white/cream residue remained each Teflon vial was capped and left in storage ready for TIMS filament loading. Finally, any used columns were emptied of resin and rinsed with DI water, dried and returned to storage in 1 M HNO3 acid.

A.2.1.6 TIMS Filaments and Analysis

Rubidium filaments were prepared prior to sample loading and TIMS (Thermal Ionization Mass Spectrometry) Sr analysis. Older filaments, after being stripped of used rubidium, were first recycled and cleaned using AR grade acids (e.g. 6M HNO3) and an electrical grinder. These filaments were then rewelded with new rubidium strips and placed in a degasser chamber (Isotox D60). Care was taken to not contaminate the filaments before and after placement in the TIMS (Isotopx Phoenix) so gloves were used throughout the entire process. Samples prepared previously during Sr Micro Bio-Spin column procedure were taken out of storage and carefully loaded on to the rubidium filaments. Each filament was subjected to an electrical current from a power supply (GW Instek PSP - 405) and loaded with 1 uL of 1M H3PO4 and evaporated at ~.5A, loaded with .5 uL of Bircks Solution evaporated, loaded with samples in 1 uL of Bircks solution and dried at ~5A. The filaments were then placed into the TIMS and analysed overnight. Filaments for water, leaf and soil samples were prepared in separate instances and analysed in groupings based on scheduling and availability. Each analysis run was accompanied with a spiked blank, TIMS standard and seawater standard (IAPSO). Procedural blanks were required each run and sample type e.g. water sources, leaf material and soil.

A.2.2 δD & δO

A.2.2.1 Cryogenic Distillation

Due to the unavailability of equipment an extraction apparatus was designed and built for cryogenic distillation procedures. The distillation apparatus was required to be able to liberate xylem water and soil pore water for collection and analysis. This was achieved through a series of components that included a hotplate, ~20 m of 6mm vinyl tubing, Styrofoam box, dry ice, ten-valve vacuum manifold, suction flask and vacuum pump. The soil and stem samples were placed into a collection of 50 ml centrifuge tubes. These centrifuge tubes were then positioned on a hotplate. Each centrifuge tube had its lid drilled and inserted with the end of a 6 mm vinyl tubing, sealing any excess gap with a hot glue gun – inside and out. The ends of the vinyl tubing were then attached to the vacuum manifold which was subsequently connected to a suction flask and then connected between the suction flask and a vacuum pump to prevent water vapour entering said pump. Dry ice was then loaded into the Styrofoam box. Prior to the previous step the vinyl tubing in the Styrofoam box was coiled to maximize surface area contact with the dry ice. It was made sure that the system remain closed and maintained a constant low pressure vacuum to keep suction. The system was kept in a fume-hood and covered to slow the evaporation of the loaded dry ice. The hot plate was set to 100 c and samples were left on the hotplate for 3-4 days. Dry ice was replaced periodically until the final day. The coiled tubing was then taken out of the box and left to thaw. The centrifuge tubes were replaced with empty ones and attached to lids. Water was then collected after the vacuum pump had been turned off and the tubing had been straightened and left to reach room temperature. This process was done twice using the same tubing across 16 total samples.

A.2.2.2 Picarro Analysis and Preparation

Water samples requiring δH and δO analysis were taken to Flinder's Analytical lab to be measured by a Picarro Water Isotope Analyser. Sample preparation, exempting soil and stem water samples, involved discarding 3 ml of water to reduce fractionation effects. All samples were filtered prior to analysis to remove any particulate matter that would block the Picarro syringe(s) and contaminate results. It is to be noted that lake water samples had to be centrifuged beforehand to separate disolved contents for filtering and analysis because the samples had a high content of dissolved solids. Sample filtering involved injecting samples into a 25 mm diameter GS-TEK syringe filter before being transferred into 2 mL HPLC Teflon lined open-hole capped vials. 100µL of headspace was left empty to prevent over pressurization during analysis. Sample vials were then placed into the machine alongside a set standards. Standards used in analysis were in-house laboratory standards and used to calibrate samples against. Standards used in calibration included desalinated water (δH: 0.95‰ and δO: 7.4‰), bottled water (δH: –73.8‰ and δO: -10.36‰) and a rainwater sample (δH: –52.5‰ and δO: -8.49‰). Rainwater was used as a quality control check. Starting procedure involved injecting samples 7 times with Picarro syringe with the first 3 times being decanted to reduce memory effects. At completion, results were outputted into an excel program called Picarro ChemCorrect which includes uncalibrated and calibrated ratios. Lake water, groundwater and river water samples were analysed separately to rainwater and soil water samples due to sample timing. Stem and soil samples had insufficient water for analyses and so were subsequently bulked and mixed with DI water prior to analysis. Weights for both sample water and DI water were weighed and recorded for post-analysis correction. Tap water was also taken and mixed with DI as procedural checks to validate the mass balance corrections.

A.2.2.3 Water Mass Balance

A mass balance equation (Equation: $\delta_s = \delta_{s+d} - (m_s\delta_d)/m_d$) was applied to samples that were diluted and bulked with DI water prior to analysis. Corrected calculations were derived and outputted into Excel.

A.2.2.4 Rainwater Collector

Rainwater collection involved the construction of two separate collectors, one for Sr and the other for 18O/D. Each gauge was made up of brackets, a funnel, deterrent prongs and a plastic slitted filter. Brackets, for each collector, were fastened, with cable ties, to a post outside and away from litter fall and infrastructure run-off. Bottles were then placed on brackets and fastened a second time using cable ties. Schott bottle for ^{18}O and D collection was filled with paraffin to limit kinetic fractionation. HDPE bottle for Sr collection was pre-acid washed to remove Sr contaminants.

A.3 Other

A.3.1 Water Probe Multiparameter

Water probe (HANNA HI 98194 multiparameter) was taken out in the field and used to measure parameters such as salinity, conductivity, TDS, pH and temperature. This involved turning on the probe and submerging it in the chosen water source (e.g. river, lake). Results were recorded once the numbers stabilized. Lake water and river water were measured in-field. Post-collection measurements were made in lab for groundwater and performed in a 500 ml clean beaker.

Appendix B: EXTENDED RESULTS

Table 1.3 – Table shows δ ^{18}O & D data acquired via Picarro water analysis at Flinders' Analytical lab for water source, stem water and soil samples. DESAL, EVIAN and RAIN are values acquired from in-house lab standards. Lab standards were used for every 10 samples analysed. GW = Groundwater, LW = Lake Water, RW = River Water. RaW = Rainwater.

Sample ID	Type	Site	Calibrated ^{18}O	Calibrated D	Uncalibrated ^{18}O	Uncalibrated D	Alcohols
Dummy	Blank		0.87	6.45	0.06	0.46	
DESAL	Standard		0.95	7.40	0.05	0.23	
EVIAN	Standard		-10.36	-73.80	0.05	0.56	
RAIN	Standard		-8.61	-53.06	0.02	0.18	
TwixGW1	GW	3 & 4	-1.50	-19.48	0.02	0.13	
4GW1	GW	4	-2.57	-27.28	0.01	0.11	
3GW1	GW	3	-3.26	-29.75	0.03	0.11	
3LWM1	LW	3	5.04	21.21	0.05	0.38	
4LWC1	LW	4	8.51	39.50	0.03	0.27	
4LWC2	LW	4	8.65	40.58	0.02	0.04	
3LWM2	LW	3	5.03	21.99	0.02	0.13	
1RW3	RW	1	-1.14	-12.08	0.04	0.19	
1RW1	RW	1	-1.42	-13.75	0.04	0.21	
1RW2	RW	1	-1.43	-13.79	0.02	0.05	
DESAL	Standard		0.95	7.40	0.03	0.15	
EVIAN	Standard		-10.36	-73.80	0.05	0.67	
RAIN	Standard		-8.66	-53.42	0.05	0.36	
Dummy	Blank		-8.62	-52.81	0.09	0.56	
DESAL	Standard		0.95	7.40	0.07	0.46	
EVIAN	Standard		-10.36	-73.80	0.18	1.19	
RAIN	Standard		-8.63	-53.23	0.04	0.24	
S1000-015	Soil	1	-4.97	-30.65	0.02	0.22	3.82
S1055-070	Soil	1	-4.10	-28.56	0.06	0.47	
S1135-150	Soil	1	-4.57	-25.22	0.04	0.25	
S4000-015	Soil	4	-3.47	-33.00	0.03	0.10	
S4055-070	Soil	4	-4.41	-30.10	0.07	0.48	
S4135-150	Soil	4	-10.73	-75.43	0.03	0.31	

T106	Leaf	1	-4.50	-39.13	0.04	0.42	8.18
T100A	Leaf	1	-1.99	-24.83	0.06	0.43	
T100B	Leaf	1	-0.12	-19.18	0.04	0.35	10.38
T75	Leaf	1	-1.41	-12.07	0.05	0.58	
DESAL	Standard		0.95	7.40	0.03	0.37	
EVIAN	Standard		-10.36	-73.80	0.06	0.55	
RAIN	Standard		-8.54	-52.29	0.03	0.10	
T56	Leaf	1	-5.49	-43.09	0.07	0.53	16.84
T44	Leaf	1	-1.87	-21.81	0.04	0.39	6.86
T19	Leaf	4	-4.11	-27.78	0.06	0.52	
T16	Leaf	4	-2.24	-18.77	0.09	0.65	8.24
T11	Leaf	4	-2.22	-18.85	0.04	0.48	
T10	Leaf	4	-1.64	-23.27	0.05	0.18	
T05	Leaf	4	-2.02	-16.15	0.02	0.09	
RaW1	RaW	Calperum Station	-5.76	-38.33	0.02	0.30	
RaW3	RaW	Calperum Station	-5.66	-31.42	0.04	0.25	
TW1		B30	-1.50	-11.30	0.12	0.59	
DESAL	Standard		0.95	7.40	0.10	0.67	
EVIAN	Standard		-10.36	-73.80	0.08	0.73	
RAIN	Standard		-8.62	-53.24	0.09	0.61	
TW2		B30	-1.74	-12.53	0.08	0.45	
TW3		B30	-1.71	-10.88	0.08	0.80	
DO1			-1.45	-11.59	0.08	0.72	
DESAL	Standard		0.95	7.40	0.05	0.30	
EVIAN	Standard		-10.36	-73.80	0.13	1.14	
RAIN	Standard		-8.62	-53.17	0.09	0.60	

Table 1.5 – Table showing Global Meteoric Water Line (GMWL) equation and chosen isotope values. Values were chosen to generate a wide ranging trend for comparisons with collected data.

Point 1	0	10
Point 2	10	90
Point 3	-10	-70

Table 1.6 – Table shows water isotope values extracted from Global Network of Isotopes in River (GNIR) historical dataset(s). Murray - Lock 5 subset was chosen based on proximity to the study site. Data downloaded from http://www-naweb.iaea.org/napc/ih/IHS_resources_gnir.html

(Date)				
1989-01-15	-34.18861111	140.7786111	-2.54	-21.2

1989-04-15	-34.18861111	140.7786111	-1.73	-17
1989-06-15	-34.18861111	140.7786111	-5.41	-34.5

Table 1.7 – Data below acquired from water sources and analysed via a Water Probe Multiparameter. GW = Groundwater, LW = Lake Water, RW = River Water. * = Measured in lab

Sample ID	Type	Site	pH	DO	Conductivity (dS/m)	Resistivity (mΩ/cm)	TDS (ppmTDS)	Salinity (PSU)	Temperature (°C)
TwixGW1*	GW	3 & 4	6.93	8.87	55400	0	77600	36.07	9.95
3GW1*	GW	3	4.8	8.71	59276	0	29630	39.38	9.4
4GW1*	GW	4	6.1	8.4	70420	0	35300	48.09	10.17
4LWC1	LW	4	8.91	11.13	1.142	n/a	721	0.73	9.36
4LWC2	LW	4	8.93	10.76	n/a	n/a	730	0.74	10.66
3LWM1	LW	3	8.02	11.63	0.563	0.018	283	0.28	12.5
3LWM2	LW	3	7.74	11.06	0.593	0.0017	296	0.29	12.54
RW3	RW	1	8.18	11.96	0.272	0.0037	136	0.13	14.93

Table 1.8 – Table shows $^{87}Sr/^{86}Sr$ data for water source samples from the first TIMS analysis run. No reruns or repeats were required. All samples fell within the expected and acceptable 2-standard error (2se) range .000000 - .000005. GW = Groundwater, LW = Lake Water, RW = River Water. * Reruns. ** Repeats

Sample ID	Type	Site	Acquire Date	Function	Mean (After)	2se	Std Dev% (After)	Std Err% (After)
T3-4GW1	GW	3 & 4	Monday 9 July 2018 3:43:10	87/86 Exp	.709527	.000003	.0023	.00023
3GW1	GW	3	Sunday 8 July 2018 19:13:59	87/86 Exp	.709477	.000003	.0022	.00022
4GW1	GW	4	Sunday 8 July 2018 20:56:14	87/86 Exp	.709574	.000003	.0018	.00019
4LWC1	LW	4	Sunday 8 July 2018 12:44:51	87/86 Exp	.710959	.000003	.0017	.00018
4LWC2	LW	4	Sunday 8 July 2018 14:13:22	87/86 Exp	.710935	.000003	.0020	.00021
3LWM1	LW	3	Sunday 8 July 2018 15:50:5	87/86 Exp	.711784	.000003	.0020	.00020
3LWM2	LW	3	Sunday 8 July 2018 17:32:30	87/86 Exp	.711904	.000005	.0033	.00033
1RW1	RW	1	Sunday 8 July 2018 22:42:26	87/86 Exp	.713749	.000003	.0021	.00022
1RW2	RW	1	Monday 9 July 2018 0:22:10	87/86 Exp	.713676	.000004	.0026	.00026
1RW3	RW	1	Monday 9 July 2018 2:5:28	87/86 Exp	.713751	.000003	.0020	.00021
IAPSO 2018-7-8	Standard		Monday 9 July 2018 5:48:46	87/86 Exp	.709256	.000002	.0016	.00016
987 2018-7-8	TIMS Standard		Monday 9 July 2018 7:33:2	87/86 Exp	.710241	.000003	.0021	.00022

Table 1.9 – Table shows soil and lea f $^{87}Sr/^{86}Sr$ data acquired from the second TIMS analysis run. Reruns during analysis were performed on samples that showed 2-standard error (2se) above >.000005. Both T106 and S1000-015 were repeated and re-analysed due to high 2se and low initial concentrations. * Reruns. ** Repeats

T05	Leaf	4	Wednesday 25 July 2018 17:0:24	87/86 Exp	.709973	.000003	.0020	.00020
T10	Leaf	4	Wednesday 25 July 2018 19:56:54	87/86 Exp	.709877	.000003	.0019	.00019
T16	Leaf	4	Wednesday 25 July 2018 23:14:25	87/86 Exp	.709952	.000004	.0030	.00029
T56	Leaf	1	Thursday 26 July 2018 4:24:12	87/86 Exp	.710596	.000004	.0029	.00029
T75	Leaf	1	Thursday 26 July 2018 6:9:15	87/86 Exp	.710500	.000003	.0019	.00020
T100	Leaf	1	Thursday 26 July 2018 7:51:10	87/86 Exp	.710388	.000003	.0020	.00020
T11*	Leaf	4	Thursday 26 July 2018 19:53:34	87/86 Exp	.709986	.000003	.0020	.00020
T19*	Leaf	4	Thursday 26 July 2018 21:33:17	87/86 Exp	.709946	.000003	.0021	.00022
T44*	Leaf	1	Thursday 26 July 2018 23:12:56	87/86 Exp	.710527	.000003	.0019	.00019
S1055-070	Soil	1	Wednesday 25 July 2018 7:26:48	87/86 Exp	.710441	.000003	.0018	.00019
S1135-155*	Soil	1	Thursday 26 July 2018 12:37:17	87/86 Exp	.710229	.000003	.0020	.00021
S4000-015	Soil	4	Thursday 26 July 2018 14:21:36	87/86 Exp	.710081	.000003	.0021	.00021
S4055-070*	Soil	4	Friday 27 July 2018 7:20:47	87/86 Exp	.709784	.000005	.0032	.00033
S4135-155*	Soil	4	Friday 27 July 2018 9:6:16	87/86 Exp	.709750	.000004	.0025	.00025
IAPSO 1	Standard		Wednesday 25 July 2018 9:9:28	87/86 Exp	.709204	.000003	.0023	.00024
987 WT 1005	TIMS Standard		Wednesday 25 July 2018 10:52:1	87/86 Exp	.710251	.000003	.0020	.00020

Table 2.1 – Listed procedural blanks for $^{87}Sr/^{86}Sr$ sample runs, associated methods (type) used and Sr concentration.

2367	Tuesday 10 July 2018 8:53:59	Water	1	31633
2368	Wednesday 25 July 2018 14:36:4	Soils	2	21159
2369	Wednesday 25 July 2018 14:44:37	Leaves	2	332822

Table 2.2 – Total used and unused endmembers and determined isotope fractions for $^{87}Sr/^{86}Sr$ and $\delta^{18}O$ used in linear mixing models. P = Parameter. F = Fraction. M = Mixture/Total value. n/a = not used/lack of data. **Equation:** $\delta_m = \delta_a f_a + \delta_b f_b + \delta_c f_c$ and $1 = f_a + f_b + f_c$

Sample ID	Tracer	Site	P1	P2	P3	F1	F2	F3	M
			Model						
S1000-015	$\delta^{18}O$	1	RW	GW	RaW	n/a	n/a	n/a	n/a
S1055-070	$\delta^{18}O$	1	RW	GW	RaW	n/a	n/a	n/a	n/a
S1135-150	$\delta^{18}O$	1	RW	GW	RaW	n/a	n/a	n/a	n/a
S4000-015	$\delta^{18}O$	4	LW	GW	RaW	n/a	n/a	n/a	n/a
S4055-070	$\delta^{18}O$	4	LW	GW	RaW	n/a	n/a	n/a	n/a
S4135-150	$\delta^{18}O$	4	LW	GW	RaW	n/a	n/a	n/a	n/a
T106	$\delta^{18}O$	1	RW	GW	RaW	n/a	n/a	n/a	n/a
T100A	$\delta^{18}O$	1	RW	GW	RaW	0.05345	0.6	0.34655	-3.518169
T100B	$\delta^{18}O$	1	RW	GW	RaW	n/a	n/a	n/a	n/a
T75	$\delta^{18}O$	1	RW	GW	RaW	n/a	n/a	n/a	n/a
T56	$\delta^{18}O$	1	RW	GW	RaW	0.050426	0.01	0.9496748	-5.49283
T44	$\delta^{18}O$	1	RW	GW	RaW	0.1	0.1611	0.7389	-4.859922
T19	$\delta^{18}O$	4	LW	GW	RaW	0.01	0.42798	0.57202	-4.314575
T16	$\delta^{18}O$	4	LW	GW	RaW	0.01	0.34955	0.65255	-4.582901
T11	$\delta^{18}O$	4	LW	GW	RaW	0.0101	0.9	0.0899	-2.662200
T10	$\delta^{18}O$	4	LW	GW	RaW	n/a	n/a	n/a	n/a
T05	$\delta^{18}O$	4	LW	GW	RaW	0.01	0.649	0.351	-3.5929144
S1000-015	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.1705	0.1	0.8295	0.711059
S1055-070	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.0239	0.15	0.8261	0.710441
S1135-150	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.002099	0.3	0.697911	0.710229
S4000-015	$^{87}Sr/^{86}Sr$	4	LW	GW	RaW	0.01	0.739	0.261	0.710081
S4055-070	$^{87}Sr/^{86}Sr$	4	LW	GW	RaW	0.01	0.769	0.231	0.709784
S4135-150	$^{87}Sr/^{86}Sr$	4	LW	GW	RaW	0.01	0.739	0.261	0.709750
T106	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.091	0.15	0.759	.710630
T100A	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.0227	0.2	0.7773	.710388
T100B	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.0227	0.2	0.7773	.710388
T75	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.05001	0.13	0.81999	.710500
T56	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.0797	0.18	0.7403	.710596
T44	$^{87}Sr/^{86}Sr$	1	RW	GW	RaW	0.0583	0.2	0.7417	.710527
T19	$^{87}Sr/^{86}Sr$	4	LW	GW	RaW	0.0411	0.6	0.3589	.709946
T16	$^{87}Sr/^{86}Sr$	4	LW	GW	RaW	0.0511	0.6	0.3489	.709952
T11	$^{87}Sr/^{86}Sr$	4	LW	GW	RaW	0.0811	0.6	0.3189	.709986
T10	$^{87}Sr/^{86}Sr$	4	LW	GW	RaW	0.0611	0.7	0.2389	.709877
T05	$^{87}Sr/^{86}Sr$	4	LW	GW	RaW	0.0711	0.6	0.3289	.709973