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RESEARCH ARTICLE

The potential for deep groundwater use by *Acacia papyrocarpa* (Western myall) in a water-limited environment

Emma K. Steggles[1] | Kate L. Holland[2] | David J. Chittleborough[1] | Samantha L. Doudle[3] | Laurence J. Clarke[4] | Jennifer R. Watling[1,5] | José M. Facelli[1]

[1] Biological Sciences, University of Adelaide, Adelaide, South Australia, Australia

[2] CSIRO Land and Water, Glen Osmond, South Australia, Australia

[3] Iluka Resources Ltd., Kent Town, South Australia, Australia

[4] Australian Centre for Ancient DNA, University of Adelaide, Adelaide, South Australia, Australia

[5] Manchester Metropolitan University, Manchester, UK

Correspondence
Emma K. Steggles, Biological Sciences, University of Adelaide, Adelaide, SA 5005, Australia.
Email: emma.steggles@adelaide.edu.au

Abstract

Knowledge regarding the use of groundwater by plants has implications for successful mine rehabilitation and revegetation programs in water-limited environments. In this study, we combined several approaches to investigate water sources used by *Acacia papyrocarpa* (Western myall) in the far west of South Australia, including stable isotopes, water potential, groundwater and soil chemistry, and root mapping techniques. Plant $\delta^{18}O$ signatures and water potentials were compared against a range of possible sources: rainwater, surface soil water (≤1 m depth), and deep groundwater (>20 m depth). Our aim was to determine whether groundwater contributed to the mix of waters used by *A. papyrocarpa*.

Overall, we found that trees did not source surface soil water (≤1 m), and probably sourced deep soil water (i.e. >1 m) rather than deep groundwater. Groundwater, however, could not be dismissed as a potential source, as root mapping showed tree roots were capable of reaching groundwater at depths >20 m, and isotope results indicated a potential contribution by groundwater to tree water use. However, low osmotic potentials and/or high acidity levels were shown to pose likely barriers to groundwater uptake, at least at the time of sampling. We conclude that because groundwater salinity and acidity are spatially variable in this region, plants with extensive root systems may be able to utilize zones of groundwater with lower salinity and pH levels. Overall, this study contributes to our limited understanding of groundwater use by trees occurring in water-limited environments where groundwater is extremely deep (>20 m depth).

KEYWORDS

rehabilitation, stable isotopes, tree water sources, water potential

1 | INTRODUCTION

Mine sites in dry and remote regions of Australia are often established in areas considered high in conservation value. Some of the immediate impacts of mining include vegetation clearance and modifications to soil physical, chemical, and biological properties (Jasper, Robson, & Abbott, 1987; Rokich, Meney, Dixon, & Sivasithamparam, 2001), as well as changes to groundwater chemistry from tailings storage facilities (Wang, Harbottle, Liu, & Xu, 2014). In general, there are legislative requirements in place for mining companies to manage their environmental impacts during extraction processes and to rehabilitate areas for the reestablishment of self-sustaining native ecosystems. The long-term success of revegetation programs requires an understanding of plant water use strategies in undisturbed areas, so that plant–soil–water relations can, as far as possible, be optimized for reestablishing sustainable plant populations (Wang, Mu, Zhang, & Zhang, 2013).

Plant water use strategies, including seasonal shifts in groundwater dependency, have been studied in a range of ecosystems including montane coniferous forests (Xu et al., 2011); karst systems (Swaffer, Holland, Doody, Li, & Hutson, 2013); and riparian systems (Holland, Tyerman, Mensforth, & Walker, 2006; Mensforth, Thorburn, Tyerman, & Walker, 1994; Thorburn, Hatton, & Walker, 1993a; Wang et al., 2013). Most research in Australia has focused on semiarid riparian ecosystems where groundwater is relatively shallow, <5 m depth, and few studies have investigated water use by trees in regions where groundwater is more than 10 m deep. One exception is the study by Zencich, Froend, Turner, and Gailitis (2002), who used stable isotope techniques (deuterium δ^2H) to identify potential water sources for two species of *Banksia* growing over groundwater that ranged in depth

from 2.5 to 30 m. Both species were shown to use groundwater at shallow depths but not at its deepest, and the authors suggest that this pattern of water use was a function of moisture availability in shallower soil horizons, root distribution, and maximum rooting depth.

The stable isotope, oxygen-18 (^{18}O), is also used to identify potential water sources used by plants. Two studies characterized $\delta^{18}O$ in deep soils of temperate semiarid regions in Australia. Allison and Hughes (1983) and Allison, Barnes, Hughes, and Leaney (1984) sampled soil water below 0.5 m at intervals down to 15 and 7 m depths, respectively. They found $\delta^{18}O$ signatures were relatively constant at depths below 3 m and ranged between −2.0 and −4.0‰ (relative to standard mean ocean water, SMOW). In contrast, soil water above 0.5 m depth was found to have positive $\delta^{18}O$ values, most likely reflecting rainwater infiltration and enrichment from evaporation.

No significant fractionation of ^{18}O has been observed during plant uptake of soil water (Barbour, 2007), and thus, the isotopic composition of xylem water should match that of water sources (Mensforth et al., 1994). However, as plants with large root systems generally source water from a range of soil locations, the resulting composition of twig water is a complex mix of isotope signatures. Consequently, multisource mass balance analyses, such as IsoSource™ (United States Environmental Protection Agency), are used to estimate proportional contributions for each possible water source and have been used in several studies (e.g., Fan, Li, Li, & Zhu, 2013, Wang et al., 2013, and Swaffer et al., 2013). The IsoSource™ model examines all possible combinations of each source contribution (0–100%) in small increments (e.g., 1–2%), and combinations that sum to the observed isotopic mixture within a small tolerance (e.g., <0.1‰) are considered to be feasible solutions (Phillips & Gregg, 2003).

In addition to $\delta^{18}O$ measurements, water potentials (Ψ) are also used to infer the accessibility of water to plants. Soil Ψ helps to identify depths in the soil profile from which roots are physically capable of extracting water. It represents the sum of soil moisture (matric potential), soil salinity (osmotic potential), and gravity. Only soil regions with higher Ψ than shoot Ψ are available to a tree at any given time (Holland et al., 2006). Shoot Ψ can be used as an indication of overall plant Ψ because water flow from roots to leaves is proportional to the root–leaf Ψ difference and to root–leaf hydraulic conductance (Cook & O'Grady, 2006). Water potentials from the saturated zone, where matric potential approximates 0 MPa (i.e., groundwater), can also be compared using osmotic potentials calculated from the chloride concentration of the water (Holland et al., 2006).

The survivorship of some plant species in arid ecosystems depends on their ability to access groundwater, which can be located at great depths (e.g. >20 m). Some tree species in arid regions are known to have roots that extend more than 50 m below the surface, for example, *Boscia albitrunca* and *Acacia erioloba* (Jennings, 1974) and *Prosopis juliflora* (Phillips, 1963). A number of forest trees have also been reported to have roots that extend below 20 m depth (Stone & Kalisz, 1991).

In this paper, we examine water use in the long-lived (250+ years) tree, *Acacia papyrocarpa* (Western myall), which has extensive lateral and vertical root systems. Lateral roots extend radially from the trunk to a distance >20 m, and recent mining activity close to our study site has revealed vertical roots 22 m below the surface. This discovery highlights a discrepancy between the root-zone depth in undisturbed areas and the much shallower depth of overburden soils (6–8 m) replaced on top of tailings in post-mine rehabilitation sites. It raises questions about potential groundwater use, with groundwater frequently present at depths ranging between 20 and 50 m, and also about how altered plant–soil–water relations may affect the long-term survival of this species in rehabilitation sites. Shallow soil profile, due to insufficient overburden volumes, is a widespread issue for mine rehabilitation across arid regions in Australia and elsewhere (Huang, Baumgartl, & Mulligan, 2012). For many species, roots are required to grow in mine tailings (fine-grained waste material), which need to be physically and hydro-geochemically stable for plant growth. It is necessary to restore physical structures and hydraulic functions across the whole rooting zone, and the complexity of this challenge often results in short-lived remediation success as soil structure and function fails to develop, leading to poor plant survival and low recruitment levels (Huang et al., 2012).

In this study, we analyzed $\delta^{18}O$ from xylem of twigs, trunks, opposing lateral roots, and taproots of *A. papyrocarpa*, as deep-rooted species with dimorphic root architecture are likely to access water from a variety of sources. Xylem $\delta^{18}O$ signatures and shoot Ψ were compared against a range of possible sources: rainwater, surface soil water at four depths ≤1 m and deep groundwater >20 m below the surface. The overall aim of our research was to determine whether groundwater contributed to the mix of waters used by *A. papyrocarpa*.

2 | METHODS

2.1 | Study site

The study site (30°50′17.99″S and 132°12′10.37″E) was located at the Jacinth-Ambrosia (JA) mine site in Yellabinna Regional Reserve, approximately 200 km northwest of Ceduna in South Australia (Figure 1). The nearest long term weather station to our study site was located at Tarcoola, which is 220 km to the east and in similar vegetation to that found at the study site. Mean monthly minimum and

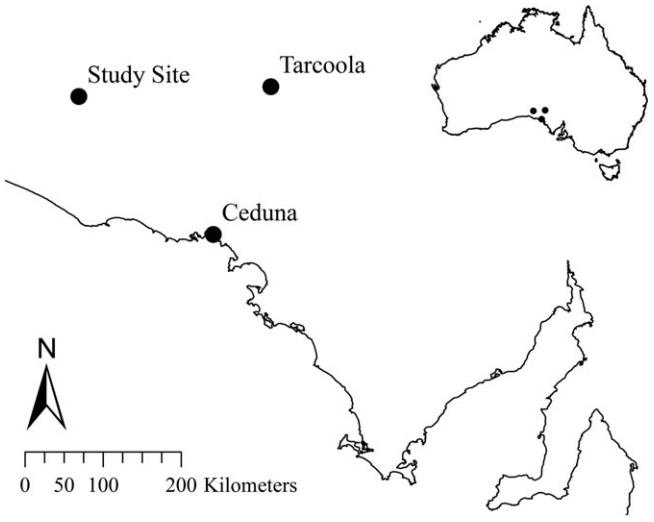

FIGURE 1 Location of the study site in Yellabinna Regional Reserve, approximately 200 km northwest of Ceduna in South Australia

maximum temperatures at Tarcoola are, respectively, 4°C and 18°C in July and 18°C and 35°C in January. Mean annual rainfall at Tarcoola is 174 mm (BOM, 2014). Rainfall is generally low and evenly spread during winter months; however, large summer rainfalls can produce floods and often occur during La Niña years (Facelli & Chesson, 2008). Rainfall was particularly low in the 6 months leading up to this study, which was carried out in mid-June (early-winter) 2012. A recently installed onsite weather station (Boztek Solutions, South Windsor, NSW) recorded only 30 mm of rain in preceding summer months and no autumn rains, which left surface soil horizons very dry.

Soils at the study site are deep calcareous sandy loams consisting of a thick layer of brown sandy loam (average 4 m) generally overlying a narrow layer of calcrete (Pratt, 2008). Non-calcareous red sandy loam extends beneath the calcrete to a depth of approximately 10 m, below which is white sand (Pratt, 2008). The physical–chemical characteristics of the brown and red sandy loam can vary, and areas of pH 9 and above are generally associated with the presence of calcium carbonate (Bean, Georgiou, & Nelson, 2012). Groundwater at the study site is restricted to fractured rock aquifers, which are heterogeneous and may have dual-porosity characteristics where groundwater is stored in preferential pathways and/or the rock matrix (Bean et al., 2012). Natural groundwater depth is generally between 20 and 50 m, and salinity levels can be as high as 68 dS/m (unpublished data).

Vegetation at the study site is sparse open woodland dominated by *A. papyrocarpa* (Fabaceae), interspersed with sandy rises and ephemeral creeks where *A. papyrocarpa* and *Eucalyptus oleosa* (Myrtaceae) co-occur. *Acacia papyrocarpa* is a long-lived tree to 10 m high, often with multiple stems and a rounded canopy that spreads outwards with age. Individuals reach maturity after approximately 75 years, and their lifespan exceeds 250 years (Ireland, 1997). The species is restricted to semiarid and arid regions in southern Australia where they form sparse open woodlands that extend across a narrow band fringing the Nullarbor Plain (Johnson & Burrows, 2001). The understory plant community is dominated by perennial chenopod shrubs and a suite of annual forbs and grasses that emerge from the soil seed bank following suitable rainfall and temperatures.

2.2 | Tree sampling for stable isotope analysis

Three trees (Myall 1, 2, and 3) were selected from an undisturbed natural stand 150 m south of the JA tailing storage facility. The maximum distance between trees was 150 m. The location was chosen because the trees were in close proximity to established monitoring bores, with maximum distance to bores <380 m. Trees were mature, of similar life form (i.e., age), and approximately 5 m in height with 3–4 main stems. We sampled one tree per day over three consecutive days in mid-June (i.e., early winter). Two opposing primary lateral roots (PR), north and south facing, were identified at the base of each tree and exposed using shovels and trowels (Figure 2). North and south aspects were chosen because of potential differences in solar radiation experienced by plant leaves and soils, which can cause differences in leaf temperature, and thus vapor pressure deficit, and also soil temperature (Maren, Karki, Prajapati, Yadav, & Shrestha, 2015). The former could influence shoot Ψ, and the latter could affect soil moisture. Primary lateral roots were generally large (approximately 20 cm diameter) and woody, often

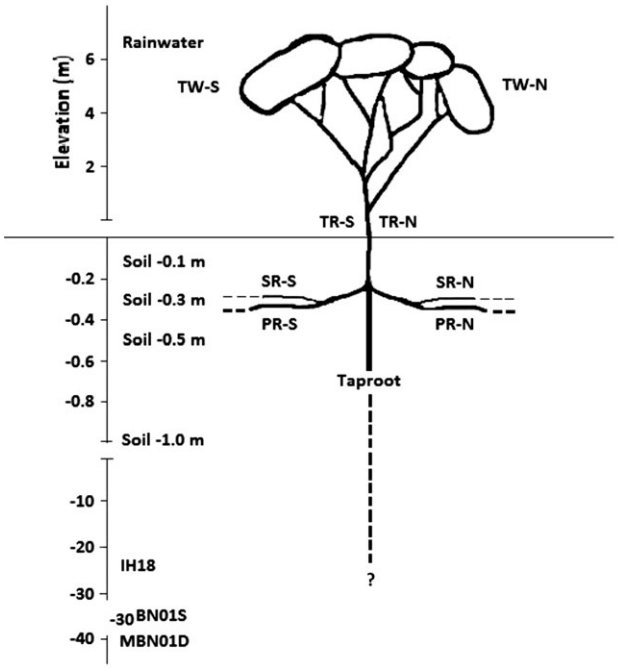

FIGURE 2 Schematic showing sampling positions for $\delta^{18}O$ analysis of *Acacia papyrocarpa* xylem water (TW = twig; TR = trunk; PR = primary lateral root; SR = secondary lateral root; N = north; S = south), soil water (soil) and groundwater (MBNO1D, MBNO1S and IH18 = groundwater monitoring bores). Three separate trees were sampled

with a secondary lateral root (SR) of smaller diameter (approximately 5 cm), that was relatively smooth-barked. Opposing SRs were only located on two of the trees (Myall 1 and 2). Selected roots were sampled within 50 cm distance from the trunk and at a depth of between 20 and 50 cm. We were able to access the taproots of Myall 1 and 2 using shovels and a small excavator; however, this was not possible for Myall 3 because access was restricted by the arrangement of its PRs.

A modified wad punch (Blackwoods, Regency Park, South Australia) and hammer were used to extract small cores of xylem tissue (approximately 1 cm^3) from PR and trunk (TR) positions. A total of 25 cores were collected from each PR and TR position and immediately immersed in kerosene in 150 ml glass jars sealed with metal lids and secured with electrical tape to prevent evaporation. Trunk samples were taken on opposite sides of the tree, matching the aspect of PRs (Figure 2).

For each canopy aspect, north and south, a single twig was cut from a healthy-looking branch (approximately 1.5 cm diameter and 20 cm long). After removing the bark, twigs were cut into 1.5 cm sections and immersed in kerosene as described above. Sections of SRs were also processed in this manner.

2.3 | Potential water sources—groundwater, rainwater, and soil water

We determined isotopic signature and Ψ of rainwater, soil water (≤1 m), and groundwater at the site (Figure 2). Rainwater was collected on the first morning of sampling. Total rainfall measured for the day was <2 mm, recorded by an onsite weather station (Boztek Solutions, South Windsor, NSW). Groundwater was collected from three monitoring bores: MBN01D (40 m depth), MBN01S (35 m depth), and

IH18 (23 m depth). Water samples were obtained by a commercial provider from monitoring bores approximately two weeks after trees and soils were sampled, following purging and bore recovery from aquifers (OTEK Practical Environmental Solutions, Adelaide, South Australia). The delay in sampling groundwater was considered acceptable, given isotope signatures were unlikely to change within a two-week period, and this was validated by similar values obtained from subsequent sampling 14 months later. Groundwater and rainwater samples were collected in triplicate and stored in glass McCartney bottles (Microteknik, Haryana, India).

To collect soil samples, a 1.2 m deep trench was excavated approximately 5 m to the west of each tree, outside the canopy edge. Soil bulk density rings (258 cm^3) were used to collect samples from the freshly exposed face of each trench at 0.1, 0.3, 0.5, and 1.0 m depths. Soil samples were transferred to 500 ml glass jars with metal lids and sealed with electrical tape to minimize evaporation.

2.4 | Isotope analyses

Azeotropic distillation (Revesz & Woods, 1990) was used to extract water from plant xylem tissue and soils. Oxygen isotope analysis was conducted by mass spectrometry as per Thorburn, Walker, and Brunel (1993b) and Brunel, Walker, Dighton, and Monteny (1997). All isotope extractions and analyses were carried out by a commercial provider (Isotope Analysis Service, CSIRO Land and Water, Waite, South Australia). IsoSource™ (US EPA) was used to determine bounds for the contributions of each potential tree water source as per Phillips and Gregg (2003). Combinations of each potential tree water source contribution were analyzed at 1.5% increments and were considered feasible within a tolerance of 0.01‰.

2.5 | EC and pH measurements

Sufficient groundwater, rainwater, and soil samples were collected to measure electrical conductivity (EC) and pH. Groundwater EC and pH were measured by a commercial provider (OTEK Practical Environmental Solutions, Adelaide, South Australia). Rainwater and soil EC and pH were analyzed with an ultrameter (Myron L Company 6PSI ultrameter II). Soil EC and pH were determined using the 1:5 soil/water method, and EC was converted to estimated EC (ECe) with a texture conversion factor as per Wetherby (2003).

2.6 | Plant shoot, groundwater and soil water potentials

Predawn shoot Ψ was measured on each sampling day using a Scholander pressure chamber (PMS Instrument Company, USA). Three replicate shoot samples (approximately 5 mm diameter) were obtained from north- and south-facing aspects of the canopy and measured immediately following collection. We present shoot Ψ means for each tree aspect. The nonparametric Mann–Whitney *U* test was chosen to analyze whether aspect influenced shoot Ψ for each tree, as data violated the homogeneity of variance assumption required for the independent samples *t*-test.

Additional soil samples were collected from each trench to measure soil Ψ at four depths: 0.1, 0.3, 0.5, and 1.0 m. Soil was collected in bulk density rings and placed into 300 ml glass jars with metal lids and sealed with electrical tape. Soil Ψ was calculated by adding together matric (Ψ_m), osmotic (Ψ_o), and gravitational (Ψ_g) pressure potentials. Matric potential was determined by the "filter paper" technique (Greacen, Walker, & Cook, 1989). The formula $\Psi_o = 0.36 \times EC \times 10^3$ was used to calculate osmotic pressure of soil solutions from EC measurements as per Allison et al. (1954). Gravimetric water content ($g\ g^{-1}$) was calculated from wet and dry weights, with soil dried at 120°C for 24 hr. Groundwater osmotic potentials were calculated as per Holland (2002). Gravitational pressure (0.098 MPa m^{-1}) was added to both soil and groundwater Ψ as per Taiz and Zeiger (2010).

2.7 | Root and soil samples collected from the mine pit

During the mining process, root and soil samples were collected from the wall and floor of the mine pit, with the purpose of increasing our understanding of natural rooting depths and *in situ* soil properties. A database was compiled, containing characteristics of each root sample (i.e., length, diameter, and surface description), the sample depth and *in situ* soil pH, EC, and ECe levels. A differential GPS (Trimble 5800™ and TSC3 controller) was used to verify the position of each set of samples, that is, latitude (x), longitude (y), and elevation (z). The original surface z value was then used to calculate the depth (m) of each sample set. Soil samples were collected from the immediate vicinity of root samples and analyzed for EC, ECe, and pH as per methods above. Several of the deepest roots were selected for DNA sequencing to identify them to species. The internal transcribed spacer 2 (ITS2) was PCR-amplified (polymerase chain reaction) using a plant-specific forward primer (ITS2P, Hugh Cross, unpublished data, contact L. Clarke for details) and ITS2 S3R (Chen et al., 2010). PCR products were Sanger sequenced using standard protocols as per Clarke, Jardine, Byrne, Shepherd, and Lowe (2012). Putative identifications for each consensus sequence were obtained by performing a local BLAST search against a reference DNA sequence database generated from plant voucher specimens from the study site.

3 | RESULTS

3.1 | Spatial variation in $\delta^{18}O$ signatures

We observed variation in isotopic signatures between trees, tree parts, and water sources (Figure 3). Isotopic signatures were similar between north and south aspects within trees (Table 1). Mean (±SEM) north and south twig signatures were −1.47‰ ± 0.13 (Myall 1), −0.84‰ ± 0.01 (Myall 2), and −0.69‰ ± 0.06 (Myall 3). Root signatures were generally negative with some exceptions; for instance, the positive signature obtained from the north-facing SR of Myall 1 suggests this root was obtaining water from a different source from other roots. North-facing PRs and SRs from Myall 2 and both opposing PRs from Myall 3 also had positive values. Signatures from rainwater and surface soils ≤1 m deep were positive, ranging from 2.19‰ to 9.70‰, reflecting rainwater infiltration and enrichment from evaporation. Groundwater signatures were variable: +0.44‰ (MBN01D), −0.98‰ (MBN01S), and −1.93‰ (IH18). This variability between groundwater sources was also detected in subsequent analyses 14 months later: +0.39‰

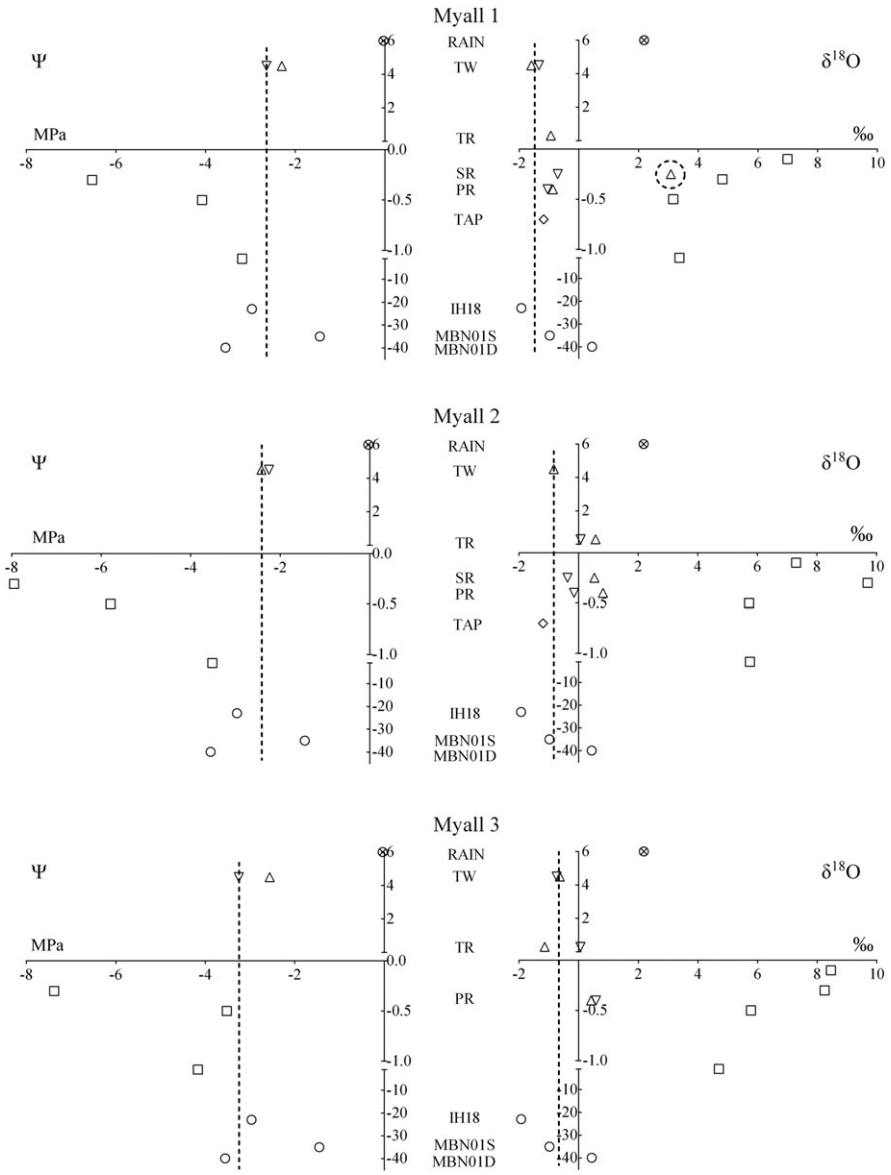

FIGURE 3 Water potential (Ψ; MPa) and $\delta^{18}O$ (‰ relative to VSMOW) results for three *A. papyrocarpa* trees (Myall 1–3) and their potential water sources. Soil Ψ from 0.1 m depths are not shown because values were > −10 MPa and beyond the capacity of trees to extract. Dotted lines represent lines of best fit for lowest shoot Ψ values and mean twig $\delta^{18}O$ signatures. The dotted circle for Myall 1 highlights the strongly positive $\delta^{18}O$ value for SR-N. TW = twig/shoot; TR = trunk; PR = primary lateral root; SR = secondary lateral root; TAP = taproot; IH18, MBNO1S and MBNO1D = groundwater monitoring bores. Symbols: triangles = TW, TR, PR & SR (△ = north aspect; ▽ = south aspect); ◇ = taproot □ = soil water; ○ = groundwater; ⊗ = rainwater

(MBN01D), −2.09‰ (MBN01S), and −2.26‰ (IH18). The decrease in $\delta^{18}O$ at MBN01S is likely due to groundwater mixing as a result of mining activities at the site (S. Doudle, pers. obs. 2013).

3.2 | Plant shoot, groundwater and soil water potentials

Shoot Ψ values were generally consistent between trees. For Myall 1, mean (± SEM) shoot Ψ (MPa) were −2.30 ± 0.35 (north) and −2.64 ± 0.09 (south). Similarly, Myall 2 Ψ were −2.42 ± 0.01 (north) and −2.25 ± 0.14 (south); and Myall 3 Ψ were −2.56 ± 0.24 (north) and −3.25 ± 0.25 (south) (Figure 3). Aspect did not influence shoot Ψ results in any of the trees ($P > 0.05$). Water potentials from groundwater sources varied: −3.56 MPa (MBN01D), −1.45 MPa (MBN01S), and −2.97 MPa (IH18), reflecting variation in EC levels (Table 2). Soil Ψ were extremely low at 0.1 m depths, ranging between −14.38 MPa (Myall 2) and −36.61 MPa (Myall 3), primarily due to low soil water content (GWC) creating low matric potentials (Table 2). Soil Ψ generally became less negative with increasing depth, ranging between −7.94 and −6.53 MPa at 0.3 m depth, and −4.16 and −3.19 MPa at 1.0 m depth (Table 2).

3.3 | Surface soils ≤1 m deep and rainwater as possible sources

IsoSource™ results indicate that for all trees examined, the 25th and 75th percentiles for possible surface soil water use ranged between 0 and 5% for mean twig water sources, indicating little or no contribution from surface soil water at the time of sampling (Table 1). Water potentials showed surface soils were too dry for trees to extract water, primarily due to low soil water content but also naturally high salinity levels (Figure 3 and Table 2). In contrast, the north-facing SR of Myall 1 had similar $\delta^{18}O$ signatures to soils at 0.5 and 1.0 m depths (Figure 3 and Table 2), suggesting the root was sourcing soil water but possibly from deeper horizons. This demonstrates an advantage of analyzing signatures from multiple positions within a tree, especially trees with extensive and deep root networks, when examining complex water source patterns that may not necessarily be detected in twig signatures alone.

Rainwater use was considered feasible for all trees examined, despite only 2 mm of rain falling on the first day of sampling. For all trees, the 25th and 75th percentiles for possible rainwater use

TABLE 1 IsoSource™ estimates of percentage water use for three *A. papyrocarpa* trees (Myall 1–3) showing 25th and 75th percentile ranges

		Percentage twig water use estimates (%)							
		Soil depth (m)				Groundwater			
Tree/ position	$\delta^{18}O$ (‰)	0.1	0.3	0.5	1.0	MBN01D	MBN01S	IH18	Rainwater
Myall 1	**(‰)**	**+6.99**	**+4.81**	**+3.16**	**+3.37**	**+0.44**	**−0.98**	**−1.93**	**+2.19**
TW-Mean	**−1.47**	0–0	0–2	0–2	0–2	0–5	3–14	*78–87*	1–4
TW-N	**−1.59**	0–0	0–2	0–2	0–2	0–5	2–11	*84–90*	1–3
TW-S	**−1.34**	0–2	0–2	0–3	0–3	2–6	3–17	*74–84*	1–4
TR-N	**−0.94**	0–2	0–3	0–5	0–5	2–9	5–26	*57–74*	1–6
TR-S	**—**	—	—	—	—	—	—	—	—
PR-N	**−0.87**	0–2	0–3	0–5	0–5	2–11	6–27	*54–72*	1–7
PR-S	**−1.05**	0–2	0–3	0–3	0–3	2–9	5–23	*62–77*	1–6
SR-N	**+3.08**	0–3	20–42	6–29	8–32	2–9	2–8	3–7	3–17
SR-S	**−0.71**	0–3	0–3	0–5	0–5	2–12	6–30	*48–68*	3–7
Taproot	**−1.18**	0–2	0–2	0–3	0–3	2–8	5–20	*68–80*	1–6
Myall 2	**(‰)**	**+7.30**	**+9.70**	**+5.72**	**+5.75**	**+0.44**	**−0.98**	**−1.93**	**+2.19**
TW-Mean	**−0.84**	0–3	0–2	0–3	0–3	2–11	6–29	*54–74*	3–7
TW-N	**−0.83**	0–3	0–2	0–3	0–3	2–11	6–29	*54–74*	3–7
TW-S	**−0.84**	0–3	0–2	0–3	0–3	2–11	6–29	*54–74*	3–7
TR-N	**+0.57**	2–6	0–5	2–8	2–8	5–23	9–38	18–45	4–15
TR-S	**+0.07**	0–5	0–3	0–6	0–6	5–20	9–39	*27–54*	3–12
PR-N	**+0.82**	0–3	2–6	2–9	2–9	6–27	9–36	14–39	4–18
PR-S	**-0.15**	0–5	0–3	0–5	0–5	3–18	9–39	*32–59*	3–12
SR-N	**+0.53**	2–6	0–5	2–8	2–8	5–23	9–39	20–47	4–15
SR-S	**−0.37**	0–3	0–3	0–5	0–5	3–15	8–36	*38–63*	3–10
Taproot	**−1.19**	0–2	0–2	0–2	0–2	2–8	5–21	*68–81*	1–6
Myall 3	**(‰)**	**+8.46**	**+8.25**	**+5.78**	**+4.7**	**+0.44**	**-0.98**	**−1.93**	**+2.19**
TW-Mean	**−0.69**	0–2	0–3	0–3	0–5	2–12	6–32	*48–69*	3–9
TW-N	**−0.63**	0–3	0–3	0–3	0–5	3–14	8–33	*47–69*	3–9
TW-S	**−0.74**	0–2	0–2	0–3	0–3	2–12	6–30	*50–71*	3–7
TR-N	**−1.14**	0–2	0–2	0–2	0–3	2–9	5–21	*65–80*	1–6
TR-S	**+0.06**	0–5	0–5	0–6	2–6	3–20	9–39	*27–54*	3–12
PR-N	**+0.42**	0–5	0–5	2–8	2–8	5–23	9–39	21–48	4–15
PR-S	**+0.58**	0–5	2–6	2–8	2–9	5–24	9–38	18–44	4–16

Estimates in excess of 50% twig water use are indicated by italic font. Stable isotopes of water sample values (‰) are in bold font. TW-mean is the mean of twig signatures from north and south aspects of each tree. Data are missing for Myall 1 TR-S due to insufficient water extracted for analysis.

TW = twig; TR = trunk; PR = primary lateral root; SR = secondary lateral root; N = north aspect; S = south aspect; MBNO1D, MBNO1S, and IH18 = groundwater monitoring bores.

were low, ranging between 1 and 9% for mean twig water sources (Table 1). For Myall 1, percentiles were similarly low for possible rainwater use in all PRs and SRs (excluding SR-N). However, percentiles from Myall 2 and 3 were higher in PRs and SRs, ranging between 3 and 18%, which may reflect a delay in the uptake of rainwater by roots and subsequent transportation to twigs. Unfortunately we cannot dismiss the possibility that rainwater has a similar $\delta^{18}O$ signature to soil water from soil horizons >1 m depth or to deep groundwater (e.g., MBN01D).

3.4 | Groundwater as potential water sources

The DNA analyses confirm that *A. papyrocarpa* roots occur 22 m below the surface, well within reach of groundwater (Figure 4). However, our results are inconclusive with regard to their use of groundwater. Water potential and salinity results suggest that trees were probably unable to extract water from MBN01D, as it was too saline (Figure 3 and Table 2). This is also reflected in IsoSource™ results from mean twig signatures, with 25th and 75th percentiles for possible MBN01D use ranging between 0 and 12% (Table 1). In contrast, Ψ results indicate that trees could extract water from MBN01S (Figure 3), yet IsoSource™ results from mean twig signatures are ambiguous, with percentiles ranging between 3 and 32% (Table 1). Only Myall 3 had the potential to extract water from IH18 (Figure 3); however, IsoSource™ results show moderate to high percentiles for possible use by all three trees examined, ranging between 48 and 87%. The low pH value (3.3) in groundwater from both IH18 and MBN01S is a likely obstacle to tree water use (Table 2). Analyses from soil samples collected alongside plant roots in the mine pit show roots occurring in soils with pH as low as 4.2 (Figure 4); however, we have no evidence of trees being able to use groundwater with pH as low as 3.3, as in IH18 and MBN01S.

TABLE 2 Salinity (EC), pH, and water potential (Ψ) of rainwater, groundwater (IH18, MBN01S, and MBN01D), and soil water (≤1 m depth) (Myall 1, 2, and 3).

Type	Sample	Depth (m)	EC dS/m	pH	Ψ MPa	Texture	ECe dS/m	GWC %
Water	Rainwater	0.0	0.7	6.6	−0.03	—	—	—
	MBN01D	40.0	68.2	6.1	−3.56	—	—	—
	MBN01S	35.0	23.9	3.3	−1.45	—	—	—
	IH18	23.0	59.0	3.3	−2.97	—	—	—
Myall 1	Soil	0.1	0.3	8.7	−27.61	SCL	2.9	2.7
	Soil	0.3	1.5	9.7	−6.53	LSCL	14.6	5.6
	Soil	0.5	2.1	9.8	−4.09	CL	20.1	7.1
	Soil	1.0	4.7	9.7	−3.19	SCL	45.1	5.6
Myall 2	Soil	0.1	0.5	8.3	−14.38	SCL	4.8	2.9
	Soil	0.3	1.0	9.7	−7.94	SCL	9.5	4.3
	Soil	0.5	1.3	9.9	−5.79	SCL	12.1	5.0
	Soil	1.0	0.6	10.1	−3.51	LSCL	5.6	2.1
Myall 3	Soil	0.1	0.6	8.7	−36.61	SCL	6.0	3.6
	Soil	0.3	1.5	9.6	−7.38	SCL	14.1	6.2
	Soil	0.5	2.5	9.9	−3.52	CL	24.2	9.9
	Soil	1.0	3.4	9.7	−4.16	SCL	32.5	6.0

Table also includes texture, estimated electrical conductivity and gravimetric water content of soils (≤1 m depth).

SCL = sandy clay loam, LSCL = light sandy clay loam, CL = clay loam.

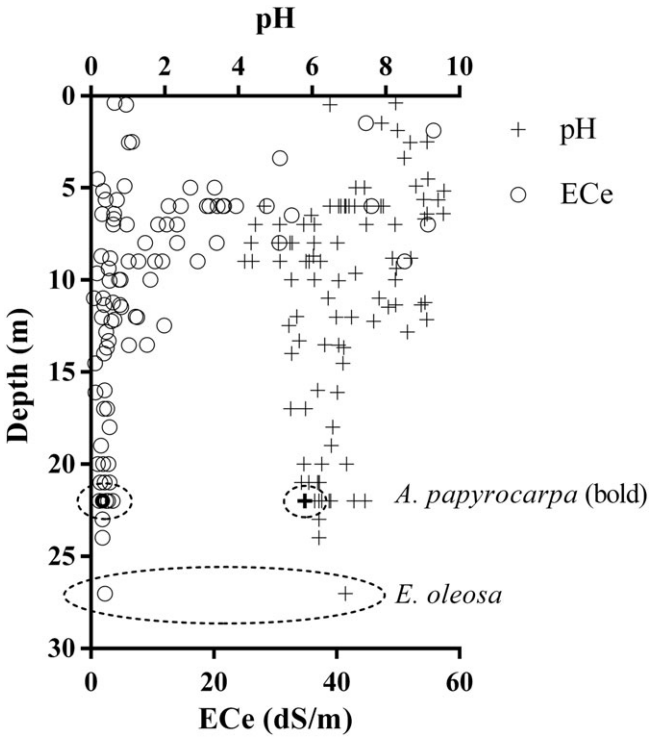

FIGURE 4 Rooting depths and the range of associated soil pH and ECe measurements collected from the mine pit. Each root sample is represented by a pH and ECe symbol. Samples associated with sandy rises and creek lines (i.e., where *Acacia papyrocarpa* co-occurs with *Eucalyptus oleosa*) are included here. A selection of roots were identified through DNA analysis, and the dotted circles highlight the maximum known rooting depths for *A. papyrocarpa* and *E. oleosa*

4 | DISCUSSION

Plants in water-limited environments with deep root systems regularly extract water from deep soil horizons and groundwater because of unreliable shallow water sources (Wang et al., 2013). Groundwater use in arid regions of Australia is often discounted due to its depth (i.e., >20 m), especially when rooting depths are unknown, and high groundwater salinity levels. Root samples collected from the JA mine pit at our study site revealed *A. papyrocarpa* roots in the vicinity of deep groundwater, prompting us to consider it as a potential water source. Our findings indicate that at the time of sampling, the use of water from deep soil horizons >1 m depth was more probable than groundwater. However, we suggest that deep groundwater use by *A. papyrocarpa* in different spatial and temporal settings is likely.

Rainwater, on the first day of sampling, contributed little to twig water mixtures, reflecting the low amount of rainfall received (<2 mm). Water potential results showed that trees were not able to extract soil water from horizons ≤1 m deep, because of the dry conditions leading up to the study. Based on the similarities observed between $\delta^{18}O$ signatures in PRs and/or SRs from all three trees, the signatures of shallow soils (≤1 m deep), and the combination of low Ψ and low pH in groundwater, we suggest that trees were likely sourcing water from deeper soil horizons (i.e., below those sampled in this study) with higher soil moisture contents.

The role of hydraulic redistribution needs to be considered here, which is the passive movement of water through xylem pathways, from wetter (high Ψ) to drier (low Ψ) regions in the soil. After rainfall, surface soil water is transported downwards into deeper soil layers where it enables the growth and survival of deep root networks. When surface soils become dry in summer or during periods of drought, water is transported upwards via hydraulic lift where it can be used to sustain surface roots. This strategy has been documented in deep-rooted species occurring in arid environments (Bleby, McElrone, & Jackson, 2010).

Given the depths at which we have observed *A. papyrocarpa* roots, the redistribution of water into deeper soil layers likely plays a critical role in the tree's water use strategies. There is minimal infiltration of rainwater into deep soil horizons (i.e., >1 m depth) at the study site, making the vertical redistribution of water through xylem pathways potentially important for this species, with the process certainly requiring further examination. A tree's dependence on water stored in deep soil horizons has implications for species reestablishment and long-

term survival in post-mine areas, particularly when considering that modified soils and tailings often have different water holding capacities and soil chemistries than those of pre-disturbed soils (Rokich et al., 2001). Potential repercussions include reduced rooting depths and restrictions to roots accessing deeper water sources in rehabilitation sites, which may compromise the ability of plants to subsist through extended dry periods. This process also has important implications for landscape hydrology and potentially the spatial distribution of understory plant species that may rely on the redistribution of water towards the surface (Burgess et al., 2001).

The potential use of groundwater by *A. papyrocarpa* is strongly suggested by the relatively high percentiles for possible IH18 groundwater use obtained from mean twig signatures in all three trees examined, ranging between 48 and 87%. However, Ψ results showed that not all trees were capable of extracting water from IH18. This discrepancy may be attributable to the timing of sampling, as the Ψ from IH18 groundwater fits within the range of predawn shoot measurements previously recorded for *A. papyrocarpa* at this site (unpublished data). Alternatively, it may indicate that trees were sourcing water from soil regions deeper than those sampled in this study. For example, we may expect that $\delta^{18}O$ signatures in deep soils, that is, outside the range we sampled, may be negative values within the vicinity of −2.0 and −4.0‰, as per Allison and Hughes (1983) and Allison et al. (1984). If so, then we cannot discount that signatures from deeper soils may be similar to those from IH18, and this may account for the high percentiles generated from IsoSource™. The characterization of $\delta^{18}O$ from deeper soil horizons is needed to confirm whether this is the case.

Although salinity levels were very high in groundwater at the study site, salt toxicity is not likely to be an obstacle to groundwater use by *A. papyrocarpa*. *Acacia* species are well known for their widespread occurrence on naturally saline soils in Australia (Craig, Bell, & Atkins, 1990), and numerous studies have demonstrated high salt tolerance in many *Acacia* species (Aswathappa, Marcar, & Thomson, 1987; Craig et al., 1990; Thomson, 1987). Soils at the study site are naturally saline, and analyses of soil samples collected from the mine pit show roots occur in soils with ECe as high as 55 dS/m (Figure 4). In glasshouse trials, Craig et al. (1990) demonstrated growth and survival of several *Acacia* species in soils irrigated with saline solution as high as EC 95 dS/m. Also, other non-*Acacia* species have been shown to use extremely saline groundwater, with several *Eucalyptus* species occurring on floodplains along the River Murray in South Australia using groundwater with EC levels up to 33 dS/m (Thorburn et al., 1993a).

Our results suggest that low Ψ and low pH are the primary obstacles to groundwater use by *A. papyrocarpa*. Previous work in arid riparian environments has shown that trees often have low transpiration rates to reduce water use and that they are generally able to extract water at very low osmotic potentials (Costelloe et al., 2008). In addition, roots have been shown to occur in soils at the study site with pH as low as 4.2 (Figure 4), suggesting a degree of acid tolerance. This is supported by work of Ashwath, Dart, Edwards, and Khanna (1995), who examined acid tolerance in *Acacia* species and found many of the 36 species examined were able to grow and fix nitrogen in soils of 4.1 pH without adverse effects. The pH value of groundwater (3.3) for both IH18 and MBN01S is still considerably lower, and thus, further investigation is needed to establish acid tolerance levels for *A. papyrocarpa*.

Overall, we cannot rule out groundwater use from this study because salinity is spatially variable and this may enable plants with extensive root systems to utilize zones of groundwater with lower salinity. Acidity too varies between different groundwater sources, reflecting the heterogeneous nature of groundwater stored within fractured rock aquifers at the study site. Previous studies show plants undergo seasonal shifts in water use in response to water availability, with many increasing their groundwater dependency when other sources are no longer available. Wang et al. (2013) examined five species including two trees, in a semiarid ecosystem in China, and found all species were highly dependent on groundwater during the dry season but reduced their dependence during the wet season. Similar shifts in groundwater dependency have been reported in a range of studies (McCole & Stern, 2007; Mensforth et al., 1994; Xu et al., 2011). Consequently, future experimental design for examining water use by *A. papyrocarpa* should consider seasonal changes in water use patterns. Having said that, our sampling occurred after a long period without rainfall, and thus at a time when trees might be expected to access groundwater.

5 | CONCLUSIONS

Water from deep soil horizons was most probably the primary water source used by *A. papyrocarpa* trees in our study, although deep groundwater could not be discounted as a potential source under different spatial and temporal settings. Further research is needed to determine pH tolerance of *A. papyrocarpa* and to characterize $\delta^{18}O$ in soil horizons >1 m depth in order to refine our understanding. Attention should also focus on potential shifts in groundwater use patterns, the role of hydraulic redistribution in water sourcing and incorporating other co-occurring deep-rooted species into analyses. Our research highlights the implications of plant water sourcing for reestablishing sustainable plant populations in disturbed areas where water is limited.

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