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

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

[1]*Biological Sciences, University of Adelaide, Adelaide, SA 5005, Australia*
[2]*Health and Life Sciences, Northumbria University, Newcastle upon Tyne, NE1 8ST, UK*
[3]*CSIRO Land and Water, PMB 2, Glen Osmond, SA 5064, Australia*
[4]*Iluka Resources Ltd., 11 Dequetteville Terrace, Kent Town, SA 5067, Australia*
[5]*Australian Centre for Ancient DNA, University of Adelaide, Adelaide, SA 5005, Australia*

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

10   Overall we found that trees sourced deep soil water rather than groundwater, although
11   groundwater could not be dismissed entirely as a potential source. Root mapping data
12   showed tree roots were capable of reaching groundwater at depths > 20 m, and isotope
13   results indicated a potential contribution by groundwater to tree water use. However, low
14   osmotic potentials and high acidity levels were shown to pose a likely barrier to water
15   uptake, at least at the time of sampling. We conclude that because groundwater salinity
16   and acidity is spatially variable in this region, plants with extensive root systems may be
17   able to utilise zones of groundwater with lower salinity and pH levels. Overall this study
18   contributes to our limited understanding of groundwater use by trees occurring in water-
19   limited environments where groundwater is extremely deep (> 20 m depth).

20   KEY WORDS   stable isotopes; tree water sources; water potential; rehabilitation

21   *Correspondence to: Emma Steggles, Biological Sciences, University of Adelaide,
22   Adelaide, SA 5005, Australia. E-mail: emma.steggles@adelaide.edu.au

23   †Current address: Antarctic Climate & Ecosystems Cooperative Research Centre,
24   University of Tasmania, Private Bag 80, Hobart, Tasmania 7001, Australia.

INTRODUCTION

25   Mine sites in dry and remote regions of Australia are often established in areas considered
26   high in conservation value. Some of the immediate impacts of mining include vegetation
27   clearance and modifications to soil physical, chemical and biological properties (Jasper *et*
28   *al.*, 1987; Rokich *et al.*, 2001), as well as changes to groundwater chemistry from tailings
29   storage facilities (Wang *et al.*, 2014). In general, there are legislative requirements in place
30   for mining companies to manage their environmental impacts during extraction processes,
31   and to rehabilitate areas for the re-establishment of self-sustaining native ecosystems. The
32   long-term success of revegetation programs requires an understanding of plant water use

33 strategies in undisturbed areas, so that conditions can, as far as possible, be optimised for
34 re-establishing sustainable plant populations (Wang *et al.*, 2013).

35 Plant water use strategies, including seasonal shifts in groundwater dependency, have been
36 studied in a range of ecosystems including montane coniferous forests (Xu *et al.*, 2011);
37 karst systems (Swaffer *et al.*, 2014); and riparian systems (Holland *et al.*, 2006; Mensforth
38 *et al.*, 1994; Thorburn *et al.*, 1993a; Wang *et al.*, 2013). Most research in Australia has
39 focussed on semi-arid riparian ecosystems where groundwater is relatively shallow, < 5 m
40 depth, and few studies have investigated water use by trees in regions where groundwater
41 is more than 10 m deep. One exception is the study by Zencich *et al.* (2002), which used
42 stable isotope techniques (deuterium δ^2H) to identify potential water sources for two
43 species of *Banksia* growing over groundwater that ranged in depth from 2.5 m to 30 m.
44 Both species were shown to use groundwater at shallow depths but not at its deepest, and
45 the authors suggested that this pattern of water use was a function of moisture availability
46 in shallower soil horizons, root distribution and maximum rooting depth.

47 The stable isotope oxygen-18 ($\delta^{18}O$) is also used to identify potential water sources used
48 by plants. Two studies characterised $\delta^{18}O$ in deep soils of temperate semi-arid regions in
49 Australia: Allison *et al.* (1983) and Allison *et al.* (1984) showed $\delta^{18}O$ signatures in soil
50 water sampled below 0.5 m depth were negative values ranging between -2.0 and -4.0 ‰
51 relative to Standard Mean Ocean Water (SMOW). Soils were examined at intervals down
52 to 15 m and 7 m depths respectively, and the results showed $\delta^{18}O$ signatures were relatively
53 constant at depths below 3 m. In contrast, soil water above 0.5 m depth was found to have
54 positive $\delta^{18}O$ values, most likely reflecting rainwater infiltration and enrichment from
55 evaporation.

56 No significant fractionation of ^{18}O has been observed during plant uptake of soil water
57 (Barbour, 2007) and thus, the isotopic composition of xylem water should match that of
58 water sources (Mensforth *et al.*, 1994). However, isolating discrete sources is not always
59 feasible because the isotopic composition of twig xylem water is generally a mixture of
60 more than one source. The redistribution of water by tree roots can also produce complex
61 water source patterns that are not necessarily discrete sources from a particular zone in the
62 soil profile. As a consequence, multi-source mass balance analyses, such as IsoSource™
63 (United States Environmental Protection Agency), are used to estimate feasible
64 proportional contributions for each possible water source, and have been used in several
65 studies i.e. Fan *et al.* (2013), Wang *et al.* (2013) and Swaffer *et al.* (2013). The
66 IsoSource™ model examines all possible combinations of each source contribution (0–
67 100%) in small increments (e.g. 1-2%), and combinations that sum to the observed isotopic
68 mixture within a small tolerance (e.g. < 0.1‰) are considered to be feasible solutions
69 (Phillips *et al.*, 2003).

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

81　The survivorship of some plant species in arid ecosystems depends on their ability to access
82　groundwater, which can be located at great depths i.e. > 20 m. Many tree species in arid
83　regions are known to have roots that extend more than 50 m below the surface, e.g. *Boscia*
84　*albitrunca* and *Acacia erioloba* (Jennings, 1974), and *Prosopis juliflora* (Phillips, 1963).
85　Stone *et al.* (1991) present data on another 11 species, primarily forest trees, with roots
86　that extend below 20 m depth.

87　In this paper we examine water use in the long-lived (250+ years) tree, *Acacia papyrocarpa*
88　(Western myall), which has extensive lateral and vertical root systems. Vertical sinker
89　roots branch from surface laterals that extend radially from the trunk to a distance > 20 m.
90　Recent mining activity at the study area has uncovered vertical roots 22 m below the
91　surface. This discovery highlights a discrepancy between the root-zone depth in
92　undisturbed areas and the much shallower depth of overburden soils (6 - 8 m) replaced on
93　top of tailings in post-mine rehabilitation sites. It raises questions about potential
94　groundwater use, with groundwater frequently present at depths ranging between 20 and
95　50 m, and also about how altered plant-soil-water relations may affect the long-term
96　survival of this species in rehabilitation sites. Shallow soil profiles due to insufficient
97　overburden volumes is a widespread issue for mine rehabilitation across arid regions in
98　Australia and elsewhere (Huang *et al.*, 2012). For many species, roots are required to grow
99　in mine tailings (fine-grained waste material) which need to be physically and hydro-
100　geochemically stable for plant growth. It is necessary to restore physical structures and
101　hydraulic functions across the whole rooting zone and the complexity of this challenge
102　often results in short-lived remediation success as soil structure and functions fail to
103　develop, leading to poor plant survival and low recruitment levels (Huang *et al.*, 2012).

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

METHODS

111　*Study site*

112　The study site (30°50'17.99"S and 132°12'10.37"E) was located at the Jacinth-Ambrosia
113　(JA) mine site in Yellabinna Regional Reserve, approximately 200 km north-west of
114　Ceduna in South Australia (Figure 1). The nearest weather station to our study site was
115　located at Tarcoola, which is 220 km to the east and in similar vegetation to that found at
116　the study site. Mean monthly minimum and maximum temperatures at Tarcoola are,
117　respectively, 4°C and 18°C in July and 18°C and 35°C in January. Mean annual rainfall
118　at Tarcoola is 174 mm (BOM, 2014). Rainfall is generally low and evenly spread during
119　winter months, however, large summer rainfalls can produce floods and often occur during
120　La Niña years (Facelli *et al.*, 2008). Rainfall was below average at the site in the 12 months
121　leading up to this study, leaving surface soil horizons very dry.

122　Soils at the study site are deep calcareous sandy loams consisting of a thick layer of brown
123　sandy loam (average 4 m) generally overlying a narrow layer of calcrete (Pratt, 2008).
124　Non-calcareous red sandy loam extends beneath the calcrete to a depth of approximately
125　10 m, below which is white sand (Pratt, 2008). The physio-chemical characteristics of the
126　brown and red sandy loam can vary and areas of pH 9 and above are generally associated

127 with the presence of calcium carbonate (Bean *et al.*, 2012). Groundwater at the mine is
128 restricted to fractured rock aquifers, which are heterogeneous and may have dual-porosity
129 characteristics where groundwater is stored in preferential pathways and/or the rock matrix
130 (Bean *et al.*, 2012). Natural groundwater depth is generally between 20 to 50 m, and
131 salinity levels can be as high as 68 dS/m (unpublished data).

132 Vegetation at the study site is sparse open woodland dominated by *A. papyrocarpa*
133 (Fabaceae), with interspersed sandy rises and creeks where *A. papyrocarpa* and *Eucalyptus*
134 *oleosa* (Myrtaceae) co-occur. *Acacia papyrocarpa* is a long-lived tree to 10 m high, often
135 with multiple stems and a rounded canopy that spreads outwards with age. Individuals
136 reach maturity after approximately 75 years and their lifespan exceeds 250 years (Ireland,
137 1997). The species is restricted to semi-arid and arid regions in southern Australia where
138 they form sparse open woodlands that extend across a narrow band fringing the Nullarbor
139 Plain (Johnson *et al.*, 2001). The understorey plant community is dominated by perennial
140 chenopod shrubs and a suite of annual forbs and grasses that emerge from the soil seed
141 bank following suitable rainfall and temperatures.

142 *Tree sampling for stable isotope analysis*

143 Three trees (Myall 1, 2 and 3) were selected at an undisturbed site 150 m south of the JA
144 tailing storage facility. The maximum distance between trees was 150 m. The location
145 was chosen because the trees were in close proximity to monitoring bores, with maximum
146 distance to bores < 380 m. Trees were of similar life form (i.e. age) and one tree was
147 sampled per day over three consecutive days in mid-June (i.e. early winter; see also Figure
148 2). Two primary opposing lateral roots (i.e. north and south facing) were identified at the
149 base of each tree and exposed using shovels and trowels. North and south aspects were
150 chosen because of potential differences in solar radiation conditions experienced by plant
151 leaves and soils (Maren *et al.*, 2015). Primary laterals were generally large (approx. 20 cm
152 diameter) and woody, often with a secondary lateral root of smaller diameter (approx. 5
153 cm) which was relatively smooth-barked. Opposing secondary laterals were located on
154 two trees (Myall 1 & 2). All roots were sampled within 50 cm distance from the trunk and
155 between 20 and 50 cm soil depths. Myall 1 and Myall 2 taproots were accessed with
156 shovels and a small excavator. It was not possible to access the taproot of Myall 3.

157 A modified wad punch (Blackwoods, Regency Park, South Australia) and hammer were
158 used to extract small cores of xylem tissue (approx. 1 cm^3) from root and trunk positions.
159 Twenty-five cores were collected at each sample position and immediately immersed in
160 kerosene in 150 mL glass jars sealed with metal lids and secured with electrical tape to
161 prevent evaporation. Similarly, tree trunks were sampled using a wad punch on opposite
162 sides of the tree, matching the aspect of lateral roots.

163 Twigs (approximately 15 mm diameter) were collected from north and south aspects of
164 each tree canopy. Bark was removed from twigs and twig lengths (approximately 200
165 mm) were cut into 15 mm sections and immersed in kerosene as described above. Small
166 secondary roots were also processed in this manner.

167 *Potential water sources – groundwater, rainwater and soil water*

168 We examined isotopic signatures and Ψs from rainwater, soil water and groundwater at the
169 site (Figure 2). Rainwater was collected on the first morning of sampling. Total rainfall
170 measured for the day was < 2 mm, recorded by an onsite weather station. Groundwater
171 was examined from three monitoring bores: MBN01D (40 m depth); MBN01S (35 m
172 depth); and IH18 (23 m depth). Water samples were obtained from monitoring bores
173 approximately two weeks after trees and soils were sampled, following purging and bore

174 recovery of aquifers by a commercial provider (OTEK Practical Environmental Solutions,
175 Adelaide, South Australia). The delay in sampling groundwater was considered acceptable
176 given isotope signatures were unlikely to change within a two week period, and this was
177 validated by similar values obtained in subsequent sampling done 14 months later.
178 Groundwater and rainwater samples were collected in triplicate and stored in glass
179 McCartney bottles (Microteknik, Haryana, India).

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

185 *Isotope analyses*

186 Azeotropic distillation (Revesz *et al.*, 1990) was used to extract water from plant xylem
187 tissue and soils. Analysis of $\delta\,^{18}O$ was conducted with mass spectrometry as per Thorburn
188 et al. (1993b) and Brunel et al. (1997). All isotope extractions and analyses were carried
189 out by a commercial provider (Isotope Analysis Service, CSIRO Land and Water, Waite,
190 South Australia). IsoSource™ (US EPA) was used to determine bounds for the
191 contributions of each source as per Phillips *et al.* (2003). Combinations of each source
192 contribution were analysed at 1.5% increments and were considered feasible within a
193 tolerance of 0.01‰.

194 *EC and pH measurements*

195 Additional groundwater, rainwater and soil samples were collected concurrently to
196 measure electrical conductivity ($EC_{1:5}$) and pH_{water}. Groundwater EC and pH were
197 measured by a commercial provider (OTEK Practical Environmental Solutions, Adelaide,
198 South Australia). Rainwater and soil EC and pH were analysed with an ultrameter (Myron
199 L Company 6PSI ultrameter II). Soil EC and pH was determined using the 1:5 soil/water
200 method and converted to $ECe_{1:5}$ with a texture conversion factor as per Wetherby (2003).

201 *Plant shoot, groundwater and soil water potentials*

202 Pre-dawn shoot Ψs were measured on each sampling day using a Scholander pressure
203 chamber (PMS Instrument Company, USA). Three replicate shoot samples
204 (approximately 5 mm diameter) were obtained from north and south-facing aspects of the
205 canopy and measured immediately following collection. Total means of shoot Ψs are
206 presented for each tree in our results.

207 Additional soil samples were collected from each trench to measure soil Ψs at four depths:
208 0.1, 0.3, 0.5 and 1.0 m. Soil was collected in bulk density rings and placed into 300 mL
209 glass jars with metal lids and sealed with electrical tape. Total soil Ψ was calculated by
210 adding together matric (P_m), osmotic (P_o) and gravitational (P_g) pressure potentials. Matric
211 potential was determined by the 'filter paper' technique (Greacen *et al.*, 1989). The
212 relation $P_o = O.36 \times EC \times 10^3$ was used to calculate osmotic pressure of soil solutions from
213 EC measurements as per Allison *et al.* (1954). Gravimetric water content (g g^{-1}) was
214 calculated from wet and dry weights, with soil dried at 120°C for 24 hours. Groundwater
215 osmotic potentials were calculated as per Holland (2002). Gravitational pressure (0.098
216 MPa m^{-1}) was added to both soil and groundwater Ψs as per Taiz *et al.* (2010).

217 *Root and soil samples collected from the mine pit*

218 Collections of root and soil samples were made opportunely throughout the mining
219 process, from the wall and floor of the pit. Soil samples were collected from the immediate

220 vicinity of root samples and analysed for EC, ECe and pH as per method above. A
221 differential GPS (Trimble 5800™ and TSC3 controller) was used to verify the position of
222 each set of samples i.e. latitude (x), longitude (y) and elevation (z). The original surface z
223 value was then used to calculate the depth of each sample set. Several roots were selected
224 for DNA sequencing to identify the species. The internal transcribed spacer 2 (ITS2) was
225 PCR-amplified using a plant-specific forward primer (ITS2P, Hugh Cross, unpublished
226 data, contact L. Clarke for details) and ITS2 S3R (Chen *et al.*, 2010). PCR products were
227 Sanger sequenced using standard protocols as per Clarke *et al.* (2012). Putative
228 identifications for each consensus sequence were obtained by performing a local BLAST
229 search against a reference DNA sequence database generated from plant voucher
230 specimens from the study site.

RESULTS

231 *Spatial variation in $\delta^{18}O$ signatures*

232 We observed variation in isotope signatures between trees, tree parts and water sources
233 (Figure 3). There was no significant difference between north and south twig signatures
234 within trees. Mean (±SEM) north and south twig signatures were: -1.47 ‰ ± 0.13 (Myall
235 1); -0.84 ‰ ± 0.01 (Myall 2); and -0.69 ‰ ± 0.06 (Myall 3) (Table 1). Twig $\delta^{18}O$ values
236 were similar to taproot signatures in Myall 1 and Myall 2 (the taproot was not sampled in
237 Myall 3). Root signatures were generally negative with some exceptions, for instance the
238 positive signature obtained from the north-facing secondary root of Myall 1, suggests this
239 root was sourcing water differently from other roots. North-facing primary and secondary
240 roots from Myall 2 and both opposing primary roots from Myall 3 also had positive values.
241 Signatures from rainwater and surface soils ≤ 1 m deep were positive, ranging from 2.19
242 ‰ to 9.70 ‰, reflecting rainwater infiltration and enrichment from evaporation.
243 Groundwater signatures were variable: +0.44 ‰ (MBN01D); -0.98 ‰ (MBN01S); and -
244 1.93 ‰ (IH18). This variability between groundwater sources was also detected in
245 subsequent analyses 14 months later: +0.39 ‰ (MBN01D); -2.09 ‰ (MBN01S); and -2.26
246 ‰ (IH18). The decrease in $\delta^{18}O$ at MBN01S is likely attributable to groundwater mixing
247 as a result of mining activities at the site (S. Doudle, pers. com. 2013).

248 *Surface soils ≤ 1 m deep and rainwater as possible sources*

249 IsoSource™ results indicate that for all trees examined, the 25th and 75th percentiles for
250 possible surface soil water use ranged between 0 and 5% for mean twig water sources,
251 indicating little or no contribution from surface soil water at the time of sampling (Table
252 1). Water potentials showed surface soils were too dry for trees to extract water (Figure
253 3), as a consequence of low soil water contents and naturally high salinity levels (Table 2).
254 In contrast, there was a relatively high contribution from soil water detected in the north-
255 facing secondary root of Myall 1 (6-29% at 0.5 m and 8-32% at 1.0 m depth), suggesting
256 the root was sourcing soil water at > 1 m depth with similar $\delta^{18}O$ signatures to shallower
257 horizons and a higher soil moisture content. This demonstrates an advantage of analysing
258 signatures from multiple positions within a tree, especially trees with extensive and deep
259 root networks, when examining complex water source patterns that may not necessarily be
260 detected in twig signatures alone.

261 Rainwater use was considered feasible for all trees examined, despite only 2 mm of rain
262 falling on the first day of sampling. For all trees, the 25th and 75th percentiles for possible
263 rainwater use was low, ranging between 1 and 9 % for mean twig water sources (Table 1).
264 For Myall 1, percentiles were similarly low for possible rainwater use in all primary and
265 secondary roots (excluding SR-N). However, percentiles from Myall 2 and Myall 3 were

266 higher in primary and secondary roots, ranging between 3 and 18%, which may reflect a
267 delay in the uptake of rainwater by roots and subsequent transportation to twigs. However,
268 we cannot dismiss the possibility that rainwater has a similar $\delta^{18}O$ signature to soil water
269 from soil horizons > 1 m depth or to deep groundwater (e.g. MBN01D).

270 *Groundwater as potential water sources*

271 Overall our results are inconclusive with regards to groundwater use. Water potential and
272 salinity results suggest that trees were probably unable to extract water from MBN01D, as
273 it was too saline (Figure 3 and Table 2). This was reflected in IsoSource™ results from
274 mean twig signatures, with 25th and 75th percentiles for possible MBN01D use ranging
275 between 0 and 12% (Table 1). Water potential results indicate that trees were able to
276 extract water from MBN01S, however IsoSource™ results from mean twig signatures are
277 ambiguous, with percentiles ranging between 0 to 32% (Figure 3 and Table 2). Although
278 Ψ results suggest that trees were unable to extract water from IH18 (Figure 3), IsoSource™
279 results present moderate to high percentiles for possible use, ranging between 48 and 87%.
280 The low pH (3.3) in groundwater from both IH18 and MBN01S is a likely obstacle to tree
281 water use (Table 2). Analyses from soil samples collected alongside plant roots in the
282 mine pit show roots occurring in soils with pH as low as 4.2 (Figure 4); however, it is
283 uncertain whether trees could use groundwater with pH as low as 3.3, as in IH18 and
284 MBN01S.

DISCUSSION

285 Plants in water-limited environments with deep root systems regularly extract water from
286 deep soil horizons and groundwater because of a lack of reliable shallow water sources
287 (Wang *et al.*, 2013). Groundwater use in arid regions of Australia is often discounted due
288 to depth (i.e. > 20 m), especially when rooting depths are unknown, and high groundwater
289 salinity levels. Root samples collected from the JA mine pit revealed *A. papyrocarpa* roots
290 in the vicinity of deep groundwater, prompting us to consider it as a potential water source.
291 Our findings indicate that at the time of sampling, the use of water from deep soil horizons
292 > 1 m depth, was more probable than groundwater. However, we suggest that deep
293 groundwater use by *A. papyrocarpa* in different spatial and temporal settings is likely.

294 Rainwater, on the first day of sampling, contributed little to twig water mixtures and this
295 reflects the low amount of rainfall received (< 2 mm). Water potential results showed that
296 trees were not able to extract soil water from horizons ≤ 1 m deep, and this reflects the dry
297 conditions in the shallow soil horizons at the study site. Thus it follows that the similarities
298 observed between $\delta^{18}O$ signatures in primary and/or secondary roots from all three trees
299 examined, and those signatures of shallow soils ≤ 1 m deep, suggest that trees were likely
300 sourcing water from deeper soil horizons (i.e. below those sampled in this study) with
301 higher soil moisture contents.

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

310 Given the depths at which we have observed *A. papyrocarpa* roots, the redistribution of
311 water into deeper soil layers likely plays a critical role in the tree's water use strategies.
312 There is minimal infiltration of rainwater into deep soil horizons (i.e. > 1 m depth) at the
313 study site, making the vertical redistribution of water through xylem pathways potentially
314 important for this species, with the process certainly requiring further examination. A
315 tree's dependence on water stored in deep soil horizons has implications for species re-
316 establishment and long-term survival in post-mine areas, particularly when considering
317 modified soils and tailings often have different water holding capacities and soil
318 chemistries than those of pre-disturbed soils (Rokich *et al.*, 2001). Potential repercussions
319 are reduced rooting depths and restrictions to roots accessing deeper water sources in
320 rehabilitation sites, which may compromise the ability of plants to subsist through
321 extended dry periods. This process also has important implications for landscape
322 hydrology and potentially the spatial distribution of understory plant species that may rely
323 on the redistribution of water towards the surface (Burgess *et al.*, 2001).

324 The potential use of groundwater by *A. papyrocarpa*, is strongly suggested by the relatively
325 high percentiles for possible IH18 groundwater use obtained from mean twig signatures in
326 all three trees examined, ranging between 48 and 87%. However, Ψ results showed that
327 trees were not capable of extracting water from IH18. This discrepancy may be attributable
328 to the timing of sampling, as the Ψ from IH18 groundwater fits within the range of predawn
329 shoot measurements previously recorded for *A. papyrocarpa* at this site (unpublished data).
330 Alternatively, it may indicate that trees were sourcing water from soil regions deeper than
331 were sampled in this study. For example, we may expect that $\delta^{18}O$ signatures in deep soils,
332 i.e. outside the range we sampled, may be negative values within the vicinity of -2.0 and -
333 4.0 ‰, as per Allison *et al.* (1983) and Allison *et al.* (1984). If so, then we cannot discount
334 that signatures from deeper soils may be similar to the groundwater value recorded from
335 IH18, and this may account for the high percentiles generated from IsoSource™. The
336 characterisation of $\delta^{18}O$ from deeper soil horizons is needed to confirm whether this is the
337 case.

338 Although salinity levels were very high in groundwater at the study site, salt toxicity is not
339 likely to be an obstacle to groundwater use by *A. papyrocarpa*. *Acacia* species are well
340 known for their widespread occurrence on naturally saline soils in Australia (Craig *et al.*,
341 1990) and numerous studies have demonstrated high salt tolerance in many species
342 (Aswathappa *et al.*, 1987; Craig *et al.*, 1990; Thomson, 1987). Soils at the study site are
343 naturally saline, and analyses of soil samples collected from the mine pit show roots occur
344 in soils with ECe as high as 55 dS/m (Figure 4). In glasshouse trials, Craig *et al.* (1990)
345 demonstrated growth and survival of several *Acacia* species in soils irrigated with saline
346 solution as high as EC 95 dS/m. Also, other non-*Acacia* species have been shown to use
347 extremely saline groundwater, with several *Eucalyptus* species occurring on floodplains
348 along the River Murray in South Australia using groundwater with EC levels up to 33 dS/m
349 (Thorburn *et al.*, 1993a).

350 Our results suggest that low Ψs and/or low pH are the primary obstacles to groundwater
351 use by *A. papyrocarpa*. However, previous work in arid riparian environments has shown
352 that trees tolerate high soil and groundwater salinities by having low transpiration rates
353 which reduces water use, and that they are generally able to extract water at very low
354 osmotic potentials (Costelloe *et al.*, 2008). In addition, roots have been shown to occur in
355 soils at the study site with pH as low as 4.2 (Figure 4), suggesting high acid tolerance. This
356 is supported by work of Ashwath *et al.* (1995) who examined acid tolerance in *Acacia*
357 species and found many of the 36 species examined were able to grow and fixing nitrogen
358 in soils of 4.1 pH$_{water}$ without adverse effects. The groundwater pH value (3.3) for both

359 IH18 and MBN01S groundwater, is still considerably lower than known plant thresholds,
360 and thus further investigation is needed to establish acid tolerance levels for *A.*
361 *papyrocarpa*.

362 Overall, we cannot rule out groundwater use from this study because soil salinity is
363 spatially variable and this may enable plants with extensive root systems to utilise zones
364 of groundwater with lower salinity (Craig *et al.*, 1990). Acidity too, varies between
365 different groundwater sources. Previous studies show plants undergo seasonal shifts in
366 water use in response to water availability, with many increasing their groundwater
367 dependency when other sources are no longer available. Wang *et al.* (2013) examined five
368 species including two trees, in a semi-arid ecosystem in China, and found all species were
369 highly dependent on groundwater during the dry season but reduced their dependence
370 during the wet season. Similar shifts in groundwater dependency have been reported in a
371 range of studies (McCole *et al.*, 2007; Mensforth *et al.*, 1994; Xu *et al.*, 2011).
372 Consequently, future experimental design for examining water use by *A. papyrocarpa*
373 should consider seasonal changes in water use patterns through the inclusion of multiple
374 sampling times.

CONCLUSIONS

375 Water from deep soil horizons was most probably the primary water source used by *A.*
376 *papyrocarpa* trees in our study, although deep groundwater could not be discounted as a
377 potential source under different spatial and temporal settings. Further research is needed
378 to determine pH tolerance of *A. papyrocarpa* and to characterise $\delta^{18}O$ in soil horizons >
379 1m depth, in order to refine our understanding of water sources. Attention should also
380 focus on potential shifts in groundwater use patterns, the role of hydraulic redistribution in
381 water sourcing and incorporating other co-occurring deep-rooted species into analyses.
382 Our research highlights the implications of plant water sourcing for re-establishing
383 sustainable plant populations in disturbed areas where water is limited.

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390

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Table 1 IsoSource™ estimates of percentage water use for three *A. papyrocarpa* trees (Myall 1-3) showing 25th and 75th percentile ranges. The mean (±SEM) of north and south twig signatures were used for analyses: -1.47 ‰ ± 0.13 (Myall 1); -0.84 ‰ ± 0.01 (Myall 2); and -0.69 ‰ ± 0.06 (Myall 3). TR = trunk; PR = primary root; SR = secondary root; N = north aspect; S = south aspect; MBNO1D, MBNO1S & IH18 = groundwater monitoring bores. Data is missing for Myall 1 TR-S due to insufficient water extracted for analysis.

Tree/ position	$\delta^{18}O$	Soil depth (m)				Groundwater			Rain-water
		0.1	0.3	0.5	1.0	MBN 01D	MBN 01S	IH18	
MYALL 1	‰	+6.99	+4.81	+3.16	+3.37	+0.44	-0.98	-1.93	+2.19
Twigs	-1.47	0-0	0-2	0-2	0-2	0-5	3-14	78-87	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
Twigs	-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
Twigs	-0.69	0-2	0-3	0-3	0-5	2-12	6-32	48-69	3-9
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

Percentage twig water use estimates (%)

533 Table 2 Salinity ($EC_{1:5}$) and pH_{water} of rainwater, groundwater (IH18, MBN01S & MBN01D)
534 and soil ≤ 1m depths (Myall 1, 2 & 3 soil pits), and soil texture, $ECe_{1:5}$ and gravimetric water
535 content (GWC). SCL = sandy clay loam, LSCL = light sandy clay loam, CL = clay loam.

Type	Sample	Depth (m)	$EC_{1:5}$ dS/m	pH (water)	Texture	$ECe_{1:5}$ dS/m	GWC %
Water	Rainwater	0.0	0.7	6.6	-	-	-
	IH18	23.0	59.0	3.3	-	-	-
	MBN01S	35.0	23.9	3.3	-	-	-
	MBN01D	40.0	68.2	6.1	-	-	-
Myall 1	Soil	0.1	0.3	8.7	SCL	2.9	2.7
	Soil	0.3	1.5	9.7	LSCL	14.6	5.6
	Soil	0.5	2.1	9.8	CL	20.1	7.1
	Soil	1.0	4.7	9.7	SCL	45.1	5.6
Myall 2	Soil	0.1	0.5	8.3	SCL	4.8	2.9
	Soil	0.3	1.0	9.7	SCL	9.5	4.3
	Soil	0.5	1.3	9.9	SCL	12.1	5.0
	Soil	1.0	0.6	10.1	LSCL	5.6	2.1
Myall 3	Soil	0.1	0.6	8.7	SCL	6.0	3.6
	Soil	0.3	1.5	9.6	SCL	14.1	6.2
	Soil	0.5	2.5	9.9	CL	24.2	9.9
	Soil	1.0	3.4	9.7	SCL	32.5	6.0

536

537   Figure 1 Location of the study site in Yellabinna Regional Reserve, approximately 200 km
538   north-west of Ceduna in South Australia.

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

543   Figure 3 Water potential (MPa) and $\delta^{18}O$ (‰ relative to VSMOW) results for three *A.*
544   *papyrocarpa* trees (Myall 1-3) and their potential water sources. Soil Ψs from 0.1 m depths
545   are not shown because values were more negative than -8 MPa, beyond the capacities of
546   plants to extract water. Dotted lines represent the best fit for twig/shoot values and possible
547   water sources. The dotted circle for Myall 1 highlights the strongly positive $\delta^{18}O$ value for
548   SR-N. TW = twig/shoot; TR = trunk; PR = primary root; SR = secondary root; N = north
549   aspect; S = south aspect; IH18, MBNO1S & MBNO1D = groundwater monitoring bores.
550   Symbols: crosses = xylem tissue; squares = soil water; and circles = groundwater and
551   rainwater.

552   Figure 4 A summary of root and soil samples collected from the mine pit showing rooting
553   depths and associated soil pH_{water} and $ECe_{1:5}$ measurements. Samples collected from the pit
554   beneath sandy rises and creek lines (i.e. where *A. papyrocarpa* co-occurs with *E. oleosa*) are
555   included here. Only a selection of roots have been identified through DNA analysis and the
556   dotted circles highlight the maximum known rooting depths for *A. papyrocarpa* and *E. oleosa*.