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2 **Co-ordination among leaf water relations and xylem**
3 **vulnerability to embolism of *Eucalyptus* trees growing along**
4 **a gradient of depth-to-groundwater**

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15 **Running head: Impact of groundwater depth on leaf and xylem function**

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1 **Abstract**

2 The importance of groundwater resources in arid and semi-arid areas for plant survival is well

3 documented. However, there have been few studies examining the importance and impacts of

4 groundwater availability in mesic environments. The aim of this study was to determine how

5 depth-to-groundwater (DGW) impact on leaf water relations, leaf structure and branch xylem

6 vulnerability to embolism in a mesic environment. We hypothesise that increasing depth-to-

7 groundwater results in increased resistance to drought stress and that this will be manifest

8 across leaf and branch attributes pertaining to water relations. We further investigate whether

9 there is co-ordination across leaf and branch-scale level responses to increased DGW. Four

10 species were used in this study: *Eucalyptus globoidea*, *E. piperita*, *E. sclerophylla* and *E.*

11 *sieberi*.

12 Six sites were chosen along an 11 km transect to span a range of average DGW: 2.4 m, 4.3

13 m, 5.5 m, 9.8 m, 13 m, 16.3 m and 37.5 m. Leaf water relations of trees showed less

14 sensitivity to drought stress as DGW increased. This was reflected in significantly lower leaf

15 turgor loss point, maximum osmotic potential, increased maximum turgor and a reduced leaf

16 relative water content as DGW increased. At shallow DGW sites minimum diurnal leaf water

17 potentials were generally more negative than leaf water potential at zero turgor, but the

18 reverse was observed at deep sites, indicating a larger growth potential safety margin at deep

19 sites compared to shallow sites. Leaf cell wall elasticity varied independently of DGW.

20 Xylem vulnerability to embolism was quantified as the water potential associated with 50%

21 loss of conductance (P_{50}). In both summer and winter P_{50} was significantly and negatively

22 correlated with DGW. Co-ordination between leaf and branch level responses to increase in

23 DGW was apparent, which strongly supports the conclusion that groundwater supply

24 influenced woodland structure and functional behaviour.

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Introduction

Heterogeneity in water availability is an important driver in causing variation in plant structure and function (Anderegg et al. 2013; Williams et al. 1996). Plants respond to water deficit through modification or regulation of biochemical, molecular, physiological and structural characteristics, which ultimately can result in morphological and physiological adaptations (Niinemets 2001; Zhu et al. 2004). In particular, plants adapt their leaf water relations (Ngugi et al. 2004), stomatal conductance (Lo Gullo et al. 2003) and Huber value (the ratio of leaf area to sapwood area) (Carter and White 2009) and hydraulic architecture.

The impact of water deficit (Pita and Pardos 2001) or gradients of precipitation (Santiago et al. 2004) on leaf structure and water relations have been examined in different biomes along spatially extensive environmental gradients (e.g. from arid to mesic sites), using saplings (Merchant et al. 2007) or mature trees (Mitchell et al. 2008). The potential impact of differences in depth-to-groundwater on leaf structure and leaf water relations in an environment with high annual precipitation has not, to our knowledge, been examined. In this study we examine intra- and inter-specific differences in leaf water relations, leaf structure and xylem vulnerability to embolism across a natural occurring gradient in depth to groundwater. Understanding the adaptive mechanisms underlying vegetation responses to differences in water availability is important for predicting survival and growth of plants across environmental gradients.

Osmotic adjustment is an important adaptive mechanism to protect against declining water availability (Burgess and Oakley 2006; Ngugi et al. 2003). Osmotic adjustment results in enhanced capacity for turgor maintenance (Babu et al. 1999; Clifford et al. 1998). Similarly, adjustment of cell wall elasticity (Dreyer et al. 1990; Merchant et al. 2010; Pita and Pardos

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2001) and changes in specific leaf area (SLA) also occur in response to changes in water availability. Such adjustments contribute to the maintenance of the gradients of water potential between roots and leaves thereby maintaining the flow of water from roots to leaves and allowing maintenance of turgor when plants experience water stress (Mitchell et al. 2008; Suarez 2011; White et al. 2000). In this study we examine differences in leaf water relations, as derived from pressure-volume analyses (Myers et al. 1997), of several trees species growing across a naturally occurring gradient in depth-to-groundwater.

Access to groundwater plays a key role in determining plant function and survival in groundwater dependent ecosystems (Froend and Sommer 2010; O'Grady et al. 2006a). Depth-to-groundwater also influences water relations and hydraulic properties at both leaf- (Cooper et al. 2003; Horton et al. 2001) and branch-levels (Canham et al. 2009; Froend and Drake 2006). Interactions between vegetation and groundwater tend to be more pronounced during dry seasons (Froend and Drake 2006; O'Grady et al. 2006a) or in arid rather than mesic environments. Consequently, most of the research that has examined this topic has been focused on riparian ecosystems in arid and semi-arid regions (Drake and Franks 2003; Miller et al. 2010; O'Grady et al. 2006b; Rodriguez-Iturbe et al. 2007). In contrast, the work described here examined the influence and importance of groundwater depth on functional attributes of trees at both leaf and branch level in a mesic environment. We hypothesise that even in a mesic environment, the consistent availability of groundwater at shallow depths will result in significant differences in leaf water relations and xylem vulnerability to cavitation because access to groundwater will buffer vegetation during periods of lower-than-average rainfall. In Australia, drought is a recurrent feature even in mesic environments (Eamus et al. 2006) and long periods of low soil water availability represent a significant "bottle-neck" to long-term persistence of trees (Eamus et al. 2000) and adaptation of hydraulic architecture to such periods may limit hydraulic functioning during wet periods (Eamus et al. 2001, Do et al.

2008) . Testing this hypothesis will extend our present understanding of plant responses to rainfall gradients by examining the importance of gradients in groundwater depth to hydraulic function.

During transpiration water moves under tension through the xylem, which makes xylem vulnerable to cavitation (Cochard 2002; Sperry and Saliendra 1994). The negative pressure at which cavitation occurs is an indication of tolerance to drought stress (Melcher et al. 2003). Embolism reduces xylem hydraulic conductance and subsequently limits plant water transport and carbon uptake (Awad et al. 2010; Sperry and Saliendra 1994; Taylor and Eamus 2008). Thus xylem vulnerability to embolism is an important trait to consider in understanding phenotypic plasticity (Cruiziat et al. 2002) and tolerance to drought stress (Cochard 2002). Xylem vulnerability to embolism may decrease with aridity and plants growing in arid and semi-arid environments may experience a smaller proportion of embolism compared to species occupying humid environments (Alder et al. 1996; Choat et al. 2007). However, xylem vulnerability is not always directly related to aridity and can be regulated as a species-specific trait regardless of aridity (Meinzer et al. 2009).

In this study we hypothesised that trees growing at sites with shallow groundwater are more sensitive to water stress than trees growing at sites with deep groundwater because of their long-term access to groundwater. Furthermore we hypothesise that impacts of seasonal differences in rainfall on leaf structure and water relations are larger at sites with a deep water table than sites with a shallow water table because the presence of groundwater at shallow sites acts to buffer differences in rainfall. Finally we hypothesise that the response of branch hydraulic architecture is co-ordinated with the response of leaf water relations and both are impacted by depth-to-groundwater.

1   **Methods**

2   *Site description*

3   This study was conducted between January 2010 and December 2012 at six sites located in

4   remnant native *Eucalyptus* woodland within the Kangaloon bore-field in the Upper Nepean

5   catchment, 110 km south-west of Sydney, New South Wales, Australia (between $34^o29'$ S

6    $150^o34'$ E and $34^o32'$ S $150^o37'$ E). All sites have been protected from logging since their

7   declaration as "protected areas" in the 1920's. Six sites were chosen along an 11 km transect

8   to span a range of average depth-to-groundwater: 2.4 m, 4.3 m, 9.8 m, 13 m, 16.3 m and 37.5

9   m. Depth-to-groundwater in this area was monitored by Sydney Catchment Authority (SCA)

10   on daily basis since 2006 and average DGW was estimated using this data set. Average DGW

11   fluctuated minimally ($< 10\%$) across all sites during the past six years. There were four

12   dominant species across all sites: *E. piperita, E. globoidea, E. sieberi and E. sclerophylla*

13   (Table 1). The dominant tree species were defined as those that, when summed at a site,

14   accounted for > 80 % of total tree standing basal area. During 2000-2010, the study area

15   received an annual average rainfall of 1067 mm (BOM station no. 68243,

16   http://www.bom.gov.au/). However, rainfall in 2011 and 2012 was 1561 mm and 1188 mm

17   respectively (46 % and 11 % above average). The largest average monthly rainfall occurs in

18   February (186 mm) and the smallest in August (51 mm). Average minimum temperature

19   occurs in July (2.7 ^{o}C) and average maximum temperature in January (24.3 ^{o}C) (2000-2012).

20   *Pressure-volume analyses*

21   Pressure-volume analyses were used to assess leaf water relations during late summer

22   (February and early March) and late winter (August). Within each site three trees per species

1   were selected and three terminal branches (from the upper canopy, 10-15 m height,

2   depending on tree height) were excised (leaves attached) and immediately re-cut under water

3   to remove any air emboli. The newly re-cut end of the branch was placed in water and the

4   entire branch-and-leaves covered in black plastic bags to facilitate full rehydration. Following

5   overnight rehydration one mature fully expanded leaf per branch was excised and its fresh

6   weight measured using a digital balance (0.001g resolution). The leaf was allowed to lose a

7   small amount of weight through transpiration and its water potential was measured using a

8   Scholander-type pressure chamber (Model 3000, Soil moisture Equipment Corp., Santa

9   Barbara, CA). This process was repeated several times until at least five points were obtained

10   on the linear part of the type II transform (i.e. five points beyond the point at which zero

11   turgor was attained). Pressure-volume curves were established by plotting the inverse of leaf

12   water potential $(-1/\psi)$ of each sample *versus* relative water content (pressure-volume curve

13   type II) following Eamus et al. (2006). The projected area of each leaf was measured using a

14   leaf area meter (WinDAS 3.1), and leaf dry weight determined after oven drying at 65^{0}C for

15   72 h. Leaf relative water content (RWC) was calculated as:

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$$RWC = \frac{(W_T - W_D) - (W_F - W_D)}{(W_F - W_D)} \times 100$$
(Equation 1)

17   Where W_T is turgid fresh weight, W_D is leaf dry weight and W_F is leaf fresh weight.

18   From the P-V curve leaf water potential at turgor loss point (Ψ_{TLP}), relative water content at

19   turgor loss point (RWC_{TLP}), osmotic potential at full turgor (π_{100}) and modulus of elasticity (ε)

20   were calculated according to the method of Bartlett et al. (2012).

21   *Specific leaf area*

1 Specific leaf area (SLA; $cm^2 g^{-1}$) was calculated as the ratio of leaf area (cm^2) to dry mass (g)

2 of leaves and was measured during summer and winter on all species. Six leaves per tree and

3 three trees per species were sampled. Leaf area and dry mass were determined in the same

4 way as for pressure-volume analysis.

5 *Branch xylem vulnerability*

6 Branch xylem vulnerability to embolism was determined for terminal branches from all four

7 species across four sites: 2.4 m, 4.3 m, 9.8 m and 37.5 m DGW. Maximum vessel length of

8 all species at each site was measured following Macinnis-Ng et al. (2004) and all branches

9 used for vulnerability testing were at least 10 % longer than the longest vessel length

10 recorded for each species. Measurements were made on three trees per species. Embolism

11 was induced using the bench de-hydration method in summer (Dec-Feb) and air injection

12 method in winter (Jun-Aug). The change in method occurred because the air injection system

13 is much faster and provides more precise control of water potentials than the branch drying

14 method but the air injection system was not available in the summer. Xylem vulnerability

15 curves and P50 values did not differ across the two methods of assessment (data not shown).

16 The bench de-hydration method of Prior and Eamus (1999) was applied to terminal branches

17 (between 40-70 cm length and 3-6 mm diameter) by allowing excised branches to de-hydrate

18 in air for various time periods. After allowing time for partial de-hydration, water potential of

19 two leaves on the same branch was measured using a pressure chamber (Model 3000, Soil

20 Moisture Equipment Crop., Santa Barbara, CA). Following this, and prior to measuring

21 xylem hydraulic conductance, both ends of the branches were trimmed and all leaves

22 removed under water using a sharp razor blade. Leaf scars were then sealed using glue and

23 parafilm to prevent flow through them. Hydraulic conductance was then measured using two

1   custom made vacuum chambers by measuring the rate of flow of acidified, filtered and de-

2   gassed water through the branch at four pressures (20, 30, 40 and 50 kPa). After measuring

3   the initial conductance, maximum conductance of the branch was measured after flushing

4   each branch with acidified, filtered and de-gassed water at an applied pressure of 175 kPa for

5   30 minutes.

6   The air injection method used a commercially available Digital Cavitation Chamber

7   Instrument (Model 1505D-EXP, PMS Company, Albany, USA). In the air-injection method,

8   terminal branches were inserted into the cavitation chambers after all leaves had been excised

9   and leaf scars sealed with glue and parafilm. The bark at the central part of branch (where it

10   was inserted into the cavitation chambers) was removed and the sapwood was lightly notched

11   using sharp blade to facilitate air injection into the xylem. Branches were flushed with

12   acidified, filtered and de-gassed water at a pressure of 175 kPa for 30 minutes to remove air

13   bubbles from embolised vessels. Maximum hydraulic conductance of the branch was

14   measured by measuring the rate of flow of solution through the sapwood induced by positive

15   pressure. Cavitation was then induced by increasing the air pressure inside the chamber for

16   five minutes. After releasing the pressure inside the chambers, hydraulic conductance was re-

17   measured. This process was repeated using increasing pressures within the chamber until

18   90% of maximum conductance had been lost due to embolism.

19   Having measured the maximum and initial conductance in both methods, percentage loss in

20   conductance (PLC) was calculated as (Froend and Drake 2006):

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$$PLC = \frac{k_{max} - k}{k_{max}} \times 100 \qquad \text{(Equation 2)}$$

1   Where k_{max} is the maximal conductance of the branch. Vulnerability curves were generated

2   by plotting percentage loss of hydraulic conductance (PLC) against the corresponding water

3   potential (Ψ_x). Data were fitted to an exponential-sigmoidal function to find P_{50} (Equation 3):

4    $$PLC = 100/(1+\exp[a(\psi-b)])$$ (Equation 3)

5   Where a is the gradient of the linear transformation and b is constant and Ψ is the branch
6   water potential.

Pre-dawn and daily minimum leaf water potential

8   Pre-dawn and diurnal trends of leaf water potential were measured using a Scholander-type

9   pressure chamber (Model 3000, Soil moisture Equipment Crop., Santa Barbara, CA).

10   Measurements started before sunrise and continued every 90 minutes through the day. To

11   determine whether nocturnal transpiration was affecting our estimation of pre-dawn water

12   potential, three leaves per tree were wrapped in aluminium foil and covered in a ziplock

13   plastic bag on the evening prior to measurement. On the following day (at pre-dawn),

14   wrapped and adjacent unwrapped leaves were compared. Leaves inside plastic bags were

15   expected to show higher water potential and to more closely reflect soil water potential if

16   night time transpiration was evident (Bucci et al. 2004). There were no significant differences

17   between the water potentials of bagged and unbagged leaves (data not shown). Data

18   presented here are from bagged leaves only.

19   Measurements of leaf water potential were made at four sites: 2.4 m, 4.3 m, 9.8 m and 37.5

20   m. Three leaves from each of three trees per species were sampled. To access the top canopy

21   16 m and 26 m hydraulic platforms were used. Measurements were conducted in late winter

22   (early September) and late summer (February). During summer, diurnal leaf water potential

23   measurements were not collected due to large rainfall events that prevented access to some

1   sites for a period of up to six weeks. Consequently, diurnal water potential data are presented

2   for winter only.

3   *Data analyses*

4   Normal distributions of all data were tested using the Kolmogorov-Smirnov test.

5   Homogeneity of variances was tested using Leven's test with the null hypothesis that all

6   variances were equal across all groups. The impact of DGW and season on leaf water

7   relations were investigated in each species using two-way ANOVA. Results of all species

8   within each site were then pooled to determine site averages for each trait examined and a

9   one-way ANOVA test was used to examine differences between sites as a function of DGW.

10   Where significant differences were identified *post hoc* Tukey-HSD tests were performed. The

11   relationship between DGW and PLC_{50} was determined using regression analysis. Analyses

12   were performed using IBM SPSS STATISTICS (version 19, Armonk, NY, USA).

13   Significant differences were assumed to have a probability of type II errors of less than 5 %

14   ($P<0.05$).

Results

Site-average water relations

Site-average water relations were generated by combining data for all species within each site to allow us to test the first four hypotheses. Site-average water relations displayed several trends as a function of DGW and occasionally as a function of season. First, there were significant seasonal differences in π_{100} between sites during winter (F=16.12, P<0.001) and summer (F=8.45, P<0.001). Thus, π_{100} decreased significantly as DGW increased in both seasons, and this trend was stronger in winter than summer (Table 2). Similarly, Ψ_{TLP} declined significantly in winter (F=23.06, P<0.001) and summer (F=9.04, P<0.001). In winter the shallowest (2.4 m DGW) and deepest groundwater site (37.5 m DGW) had the highest (-1.03±0.04 MPa) and lowest (-1.68±0.05 MPa) Ψ_{TLP} respectively (Table 2).

There were significant differences between sites in RWC_{TLP} during both winter (F= 12.81, P<0.001) and summer (F= 6.18, P<0.001). The lowest average RWC_{TLP} (winter = 91.83 ± 0.30 and summer = 91.70 ± 0.47; Table 2) was observed at the deepest DGW site in both seasons.

Bulk elastic modulus was significantly different between sites in both summer (F=6.05, P<0.001) and winter (F=4.42, P<0.001). However, these differences did not follow a straightforward relationship with DGW. Leaves of trees at the shallowest site (2.4 m DGW) had the largest elasticity (smallest bulk elastic modulus) in both seasons (Table 2). Specific leaf area was also significantly different between sites in both summer (F=8.64, P<0.001) and winter (F=6.89, P<0.001). With the exception of one site (16. 3 m), SLA tended to be larger at the two shallowest sites and smaller at the remaining deeper sites (Table 2).

Site average xylem vulnerability to embolism

P_{50} was significantly more negative during the summer than in winter at all sites and for all species. As DGW increased, P_{50} declined curvi-linearly, reaching a constant near a DGW of 10 m (Fig. 1). That is, there was very little change in P_{50} at sites with a DGW exceeding 10 m, whereas the majority of the decline in P_{50} occurred across the three shallowest sites (< 9.8 m). There was no significant difference between winter and summer trends in P_{50}.

Co-ordination in leaf and branch traits

A strong linear correlation was observed between Ψ_{TLP} of leaves and P_{50} of branch xylem (Fig. 2), thereby demonstrating a co-ordination in the response of leaf and xylem traits to increased DGW. As depth-to-groundwater increased, sensitivity to drought, as measured by either Ψ_{TLP} or P_{50} decreased.

During winter, pre-dawn leaf water potential (Ψ_{pd}) did not show any significant relationship with depth-to-groundwater in any species (Fig. 3a). Similarly, there were no significant differences in Ψ_{min} (i.e., midday) across sites (p=0.46, F= 0.78) or species (p=0.08, F=2.22) (Fig. 3b).

Wintertime pre-dawn leaf water potentials in each species and across all sites were well above (closer to zero) the corresponding turgor loss point (Fig. 3a). The difference between pre-dawn water potential and the water potential at zero turgor increased with increasing depth-to-groundwater (Fig. 3a). The difference between minimum leaf water potential and the water potential at zero turgor was negative for trees growing at the two shallowest sites, close to zero for the two intermediate depth-to-groundwater sites (9.8 m and 13 m) and positive at the deepest site (37.5 m) (Fig. 3b).

Species specific results for leaf water relation traits

Species specific averages of all leaf water relation traits for all four species across all sites are presented in Table two and Fig. 4 to allow investigation of the hypothesis, that differences amongst species are smaller than differences across sites. Key features for each species are presented below.

E. piperita

Both π_{100} (F= 9.46, p<0.001) and Ψ_{TLP} (F=13.56, p<0.001) decreased significantly with increase in DGW irrespective of season (Table 2). No significant differences between seasons were observed for these two traits within any sites. Relative water content at the turgor loss point (RWC_{TLP}) declined significantly across sites in winter from 95.6% at site 2.4 m DGW to 92% at site 37.5 m DGW (Table 2) but not in summer. *Post hoc* tests showed that leaves from trees on the two shallowest sites (2.4 m and 4.3 m) exhibited larger RWC_{TLP} than leaves from trees growing on the deepest water-table site (37.5 m; Table 2). Seasonality was a significant factor in explaining changes in bulk volumetric elasticity (ε) (F=6.63, p=0.01). Bulk modulus of elasticity was significantly smaller during summer than winter (p<0.05), with no DGW effects found. Specific leaf area was a function of both seasonality (F=22.79, P<0.001) and DGW (F=10.96, P<0.001). Generally during winter SLA decreased with increase in DGW from 55.7 $cm^2\,g^{-1}$ at site 2.4 m DGW to 46.0 $cm^2\,g^{-1}$ at site 9.8 m DGW.

During summer P_{50} was more negative than in winter. During winter P_{50} significantly decreased (and thus sensitivity to water stress declined) as DGW increased (F=32.47, df = 1,3; p=0.02, r^2= 0.94). P_{50} ranged from -0.66 MPa at the 2.4 m DGW to -1.12 MPa at the 37.5 m DGW site (Table 2).

E. globoidea

Results of the two-way ANOVA showed that seasonality had no impact on π_{100} or Ψ_{TLP}. Both π_{100} (F= 10.48, p<0.001) and Ψ_{TLP} (F=12.27, p<0.001) declined significantly with increasing DGW. *Post hoc* comparisons showed that sites 2.4 m and 37.5 m DGW were significantly different in both seasons and for both variables (Table 2).

RWC$_{TLP}$ varied across sites as a function of DGW only (F=11.77, p<0.001) and decreased significantly from 94.2 % at site 2.4 m DGW to 91.2% at site 37.4 m DGW. Depth-to-groundwater was the only factor that affected volumetric elastic modulus (ε) (F=6.16 p<0.001). In summer ε was significantly larger at the 9.8 m DGW site (13.49 MPa) than at the shallowest site (8.93 MPa). In winter, ε in site 9.8 m DGW was significantly larger than the site 4.3 m DGW. Furthermore, elasticity did not show any significant differences within each site between winter and summer. Specific leaf area responded only to DGW (F=35.74, P<0.001). During winter SLA at the two shallowest groundwater sites was significantly larger ($\approx$ 30%) than at the two other sites with a deeper water table. No significant differences were found in SLA between two deeper sites (9.8 m and 37.5 m DGW).

Branches of *E. globoidea* growing at the 2.4 m DGW site were more vulnerable to embolism than at the other three sites in summer (Table 2) as reflected in a less negative value for *P$_{50}$*. The relationship between *P$_{50}$* and DGW was significantly different from zero in summer (F=22.79, p= 0.04; r^2= 0.91) and *P$_{50}$* declined from -0.77 MPa to -1.52 MPa as DGW increased (Table 2).

E. seiberi

Seasonality did not affect π_{100} and Ψ_{TLP} for this species. However, DGW had a significant impact on both π_{100} (F=8.31, p=0.001) and Ψ_{TLP} (F=12.65, p<0.001). During summer neither π_{100} nor Ψ_{TLP} showed any trend as a function of DGW. However, during winter π_{100} and Ψ_{TLP} declined significantly with increasing DGW, from -0.83 to -1.25 MPa for π_{100} and from -1.17 to -1.64 MPa for Ψ_{TLP} (Table 2). During summer there were no differences among sites in RWC_{TLP}, but during winter the shallowest groundwater site had a significantly larger RWC_{TLP} compared to the other two sites at which this species was found. Depth-to-groundwater (F=5.60, P=0.006) and seasonality (F=12.95, P<0.001) both contributed significantly in variation in ε. Overall during summer there were no differences in ε between sites; however, in winter significant reductions in ε with increasing DGW were observed from 11.0 MPa at site 4.3 m DGW to 13.7 MPa at site 37.5 m DGW (F=4.52, p=0.01). Both seasonality (F=35.36, P<0.001) and DGW (F=80.28, P<0.001) had significant effects on SLA. In both seasons SLA declined with increases in DGW, and SLA was consistently smaller in summer than in winter (Table 2). *Post hoc* tests showed that site 4.3 m had significantly larger SLA than two sites with 9.8 m and 37.5 m DGW.

Trees at the deepest groundwater site (37.5 m DGW) were more resistant to cavitation (more negative P_{50}) in both seasons than trees of the same species growing at shallower groundwater sites. There was a significant positive relationship between P_{50} and depth-to-groundwater for both winter (p=0.02) and summer (p=0.02). As DGW increased the P_{50} of *E. sieberi* decreased from -0.76 MPa to -1.42 MPa in summer and from -0.74 MPa to -1.57 MPa in winter (Table 2).

E. sclerophylla

Depth-to-groundwater and seasonality both contributed significantly to variation of π_{100} and Ψ_{TLP} across sites (p<0.05). Neither π_{100} nor Ψ_{TLP} varied across sites in summer, but in winter both π_{100} (F=6.34, p<0.001) and Ψ_{TLP} (F=7.58, p<0.001) decreased with increasing DGW. At the deepest site (37.5 m), π_{100} (p<0.001) and Ψ_{TLP} (p<0.001) were significantly lower in winter compared to summer (Table 2). Relative water content at turgor loss decreased significantly as a function of DGW in winter, from 96.4% at site 2.4 m DGW to 92.4% at site 37.5 m DGW (Table 2). Only the 37.5 m DGW site exhibited a significant difference (p<0.001) between winter and summer in RWC_{TLP}. Bulk modulus of elasticity did not differ as a function of DGW in either season while seasonality resulted in significantly lower ε (F=7.18, P=0.009) during winter than summer at sites 9.8 m and 16 m DGW. As DGW increased SLA tended to decrease with the exception of the 16.3 m site, where SLA was largest compared to all other sites in both seasons. Within each site seasonal differences were not statistically significant (F=0.76, P=0.38).

There were no significant differences in P_{50} between summer and winter in *E. sclerophylla*. However, P_{50} was higher (closer to zero) at the site with 4.3 m DGW compared to the two deepest groundwater sites in both seasons. The two highest P_{50} (-0.8 MPa and -0.9 MPa) were observed in summer and winter at the 4.3 m DGW site and the lowest P_{50} were observed at 37.5 m DGW site (-1.33 MPa and -1.24 MPa summer and winter respectively; Table 2).

Discussion

The impact of water deficit (Pita and Pardos 2001) or gradients of precipitation (Santiago et al. 2004) on leaf structure and water relations have been examined in different biomes along spatially extensive environmental gradients (e.g. from arid to mesic sites), using saplings (Merchant et al. 2007) or mature trees (Mitchell et al. 2008). The potential impact of differences in depth-to-groundwater on leaf structure and leaf water relations in an environment with high annual precipitation has not, to our knowledge, been examined.

Changes in traits associated with leaf and sapwood water relations were consistent with our principle hypothesis: that trees growing on shallower groundwater sites were more sensitive to water deficit than trees growing on sites having a deep water table. Osmotic adjustment was evident by decreases in π_{100} (Fig. 4) as DGW increased and as declines in the water potential at zero turgor (Table 2). Osmotic adjustment is the accumulation of osmotically active solutes (Merchant et al. 2007; Morgan 1984) and this is expressed at both maximum and zero turgor (Bartlett et al. 2012). Osmotic adjustment was significant in explaining variation in turgor loss points (Ψ_{TLP} and RWC_{TLP}) and was observed despite the wetter than average years experienced during this study. The increase in drought resistance arising from osmotic adjustment was observed across all species as DGW increased (Fig. 4). These results are consistent with previous studies by Suarez (2011), Pita and Pardos (2001) and Tuomela (1997) where water stress was either induced experimentally or through variation in rainfall. However, the results of the present study contrast with those of Carter and White (2009) who did not find any differences in turgor loss point between sites over shallow and deep groundwater in a semi-arid region. Reductions in osmotic potential allow trees to extract water from soil with a lower water potential (Bartlett et al. 2012; Pita and Pardos 2001;

24   Wright et al. 2004), thereby maintaining transpiration and photosynthesis in the early stages

25   of water stress. We therefore conclude that osmoregulation is likely to contribute to the

26   persistence of the eucalypt trees at sites where depth-to-groundwater exceeds the rooting

27   depth of the trees, even during the wetter-than-average study year.

28   Pre-dawn leaf water potential was consistently higher (closer to zero) than the leaf water

29   potential at zero turgor for all species at all sites and the difference increased significantly

30   (from 0.8 MPa to approximately 1.5 MPa) with increasing depth-to-groundwater (Fig.

31   3a).We interpret this increases as a decrease in sensitivity to drought with increasing depth-

32   to-groundwater (i.e.an increase in growth potential safety margin; *cf* Mitchell et al. (2014))

33   because the difference between soil water potential within the root zone and the point at

34   which zero turgor declined to zero (where cell expansion is zero) was largest at sites with

35   deep groundwater. This interpretation is further supported by the fact that the difference

36   between the minimum diurnal leaf water potential and the water potential at zero turgor was

37   negative for the two shallowest sites (typically approximately -0.5 MPa) but remained

38   positive (approximately 0.2 MPa) for the deepest (37.5 m) site (Fig. 3b). This supports the

39   hypothesis that trees growing on the shallowest groundwater sites were more sensitive to

40   water deficit: they experienced minimum water potentials that were often lower than the

41   water potential at zero turgor; in contrast trees growing at the deepest sites tended to maintain

42   minimum water potentials that were higher than the water potential at zero turgor and were

43   therefore better able to maintain cellular function throughout the day. We are not aware of

44   any previous study that has demonstrated this difference in leaf water relations across a

45   gradient in DGW.

46   Differences across sites in three leaf cellular traits (π_{100}, $\varPsi_{TLP}$ and RWC_{TLP}) were larger in

47   winter compared to summer. This may reflect the much wetter-than-average summers and

48   much drier-than-average winters experienced at all sites during this study. This seasonal

49   difference supports the conclusion that depth-to-groundwater influences leaf water relation

50   traits, but we found that this effect is reduced when soil moisture supplies are abundant (e.g.

51   during the wet summer experienced during this study).

52   Drought generally results in decreased leaf elasticity (Bowman and Roberts 1985; Eamus and

53   and Narayan 1990; Pita and Pardos 2001; Prior and Eamus 1999) and this can be attributed to

54   increased cell wall thickness, reduction in cell size or both (Pita and Pardos 2001). In the

55   present study the bulk modulus of elasticity (ε) did not show a significant correlation with

56   DGW except for *E. sieberi* during winter, where an unexpected decrease in ε as DGW

57   increased, was observed. None of the species showed adjustment in elasticity across sites. We

58   therefore agree with the conclusion of Merchant et al. (2007) that *Eucalyptus* species tend to

59   use osmoregulation to withstand drought, rather than changes in elasticity. Whilst Niinemets

60   (2010) found that increased bulk modulus of elasticity was the most significant leaf-scale

61   adaptation to water deficit, Scoffoni et al. (2011) and Bartlett et al. (2012) concluded that

62   changes in the water potential at turgor loss, which is driven by osmotic adjustment, was the

63   most reliable single predictor of drought resistance. In agreement with this, we observed

64   significant declines in the turgor loss point as DGW increased, further supporting our

65   hypothesis that as DGW increased, drought resistance increased.

66   Decreases in water availability along aridity gradients can result in denser leaves and hence a

67   decrease in SLA both within and between species across sites (Niinemets 2001; Pita and

68   Pardos 2001; Santiago et al. 2004). Leaves with high density have thick cell walls and a low

69   fraction of air spaces. In the present study, trees at sites with shallower groundwater had a

70   significantly larger SLA than trees growing at sites with deeper groundwater. Decrease in

71   SLA as an adaptation to water deficit has been found for many species, including Eucalypts

72   (Merchant et al. 2007; Ngugi et al. 2003). We observed declining SLA with increased DGW,

73   and conclude that increasing DGW is equivalent to declining water availability to the trees

74   and comparable in impact to declining rainfall. This trend was more significant during winter

75   than summer and this may reflect the impact of the wetter-than-average summers that were

76   recorded during the current study and reinforces the conclusion that the impact of

77   groundwater supply on leaf water relations is smaller in wet periods compared to dry periods.

78   The P_{50} in both winter and summer decreased significantly with increased DGW. Thus, as

79   DGW increased, branch xylem exhibited a larger resistance to embolism. This supports the

80   principal hypothesis of this study, namely, that trees growing over shallow groundwater sites

81   are more sensitive to xylem cavitation than trees growing over deeper groundwater. We

82   suggest that that this is a result of consistent access to groundwater at shallow sites. Increased

83   resistance to cavitation in drier environments across and within species has been extensively

84   documented through comparative studies (Allen et al. 2010; Awad et al. 2010; Choat et al.

85   2007; Maherali et al. 2004). Pockman and Sperry (2000) compared riparian with upland trees

86   and attributed the spatially more restricted distribution of most riparian species to their lower

87   resistance to cavitation.

88   Increased resistance to xylem cavitation in arid sites compared to mesic sites is not

89   universally observed. Thus no significant differences in xylem vulnerability to drought

90   induced cavitation across mesic and xeric sites and across a gradient of rainfall have been

91   observed (Maherali and DeLucia 2000; Taylor and Eamus 2008; Van der Willigen and

92   Pammenter 1998). This independence of resistance to embolism from water availability

93   suggests that the sensitivity to drought induced cavitation for some species may be a

94   genetically controlled trait that does not display a wide amplitude of plasticity in response to

95   variations in environmental conditions (Allen et al. 2010; Pockman and Sperry 2000; Taylor

96   and Eamus 2008; Van der Willigen and Pammenter 1998). Indeed it has been suggested that

97   adaptation to drier habitats may not necessarily result in high resistance to embolism (Choat

98   et al. 2012; Miranda et al. 2010). Bucci et al. (2012) suggested that some species have

99   mechanisms to maintain high leaf water potentials despite inhabiting an environment with

100   low water availability, including isohydric stomatal behaviour, a large intrinsic sapwood

101   hydraulic conductivity and a large hydraulic capacitance.

102   Some plant traits are a better indicator of plant sensitivity to water stress than others. Leaf

103   water potential at turgor loss is recognised as a physiological measure of plant sensitivity to

104   water stress (Bartlett et al. 2012; McDowell et al. 2008). Similarly, vulnerability to xylem

105   cavitation is a critical determinant of drought tolerance (Markesteijn et al. 2011; Sperry et al.

106   2008). However, leaves are likely to be more sensitive to drought than branches (Bucci et al.

107   2012; Zhang et al. 2013) because leaves are "cheaper" organs to construct than branches. A

108   strong linear correlation between these two traits (Fig. 3) across both seasons in the present

109   study showed a co-ordination in the response of leaf and branch traits, as has been observed

110   previously in a study of eight tropical dry forest species (Brodribb et al. 2003). However, we

111   did not find that leaves were more sensitive to drought than xylem (by comparing leaf turgor

112   loss point to P_{50}). This relationship indicates that as depth-to-groundwater increased,

113   sensitivity to drought at both leaf and branch-scale decreased in parallel. The responses of all

114   species in leaf-level and branch-level to increase in DGW followed a similar pattern which

115   can show these species are responding closely to an environmental factor such as increase in

116   DGW.

117   Between species differences in leaf and branch traits was surprisingly small, as evident, for

118   example, in the small standard errors in Table 2 and figure three and the convergence of all

119 species to a single regression in figure four. Much of this convergence in behaviour may be

120 attributed to the fact that all species examined where Eucalypts, but the range of values for

121 maximum solute potential and the water potential at zero turgor, for example, is much smaller

122 than that observed in a comparison of six Eucalypt species (Merchant et al. 2007) or four

123 Eucalypt species(White et al. 2000) suggesting a strong controlling impact of the similarity of

124 climate experienced across all sites.

125 *Conclusions*

126 It was hypothesized that leaf water relations and branch xylem vulnerability to embolism

127 vary as a function of depth-to-groundwater. Despite occupying a mesic habitat, the four

128 *Eucalyptus* species examined here showed significant adjustment to differences in

129 groundwater depth, comparable to observations made along gradients in rainfall. Trees at

130 sites with shallow groundwater were more sensitive to water deficit (reached zero turgor and

131 P_{50} at a higher leaf water potential compared to trees growing at sites having deeper

132 groundwater; exhibited daily minimum leaf water potentials that were lower than the water

133 potential at zero turgor). Adjustment of bulk elastic modulus was not as significant as

134 osmotic adjustment in these species. During the very wet summer season, differences in leaf

135 water relations across sites diminished compared to differences that were evident during the

136 drier winter period. It is expected that in years with below-average rainfall differences across

137 sites would become more pronounced. A co-ordination in the response of P_{50} and turgor loss

138 point was observed across all sites and both seasons. These leaf and branch level findings

139 strongly support the conclusion that groundwater supply influenced woodland structure and

140 function. We conclude that groundwater was unavailable to these tree species when depth-to-

141 groundwater was deeper than approximately 8 - 10 m.

142   **Acknowledgement**

145

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Figure legends:

Figure 1:   Pooled P_{50} of all species measured at each site as a function of depth-to-groundwater during summer (a) and winter (b). Each symbol repsresents an individual species as following: closed circle (*E. piperita*), closed square (*E. globoidea*), closed triangle (*E. sclerophylla*) and open circle (*E. sieberi*). Curves are exponential decay functions.

Figure 2:   The relationship between leaf water potential at turgor loss point (Ψ_{TLP}) and pressure at which branches lost 50 % of their conductance (P_{50}). Each point is the mean of all species at a single site; winter black circles and summer grey circles.

Figure 3:   a) Pre-dawn leaf water potential (Ψ_{pd}, MPa; bars, n=9) and b) minimum diurnal leaf water potential (Ψ_{min}, MPa; bars, n=9) *vs* leaf water potential at turgor loss point (Ψ_{TLP}, MPa; symbols) and during winter for all dominant species growing across a depth-to-groundwater gradient.

Figure 4:   Changes in solute potential at full turgor for all species as a function of depth to groundwater for winter (closed symbols) and summer (open symbols). Each symbol represents an individual species as following: closed circle (*E. piperita*), closed square (*E. globoidea*), closed triangle (*E. sclerophylla*) and open circle (*E. sieberi*).

Table 1: Dominant species at each site. The asterisk shows the presence of the species on the corresponding site.

Species	Subgenus	Family	Average depth to GW (m)						
			2.4	4.3	5.5	9.8	13	16.3	37.5
Eucalyptus globoidea	*Eucalyptus*	Mytaceae	*	*	*	*	-	-	*
Eucalyptus piperita	*Eucalyptus*	Mytaceae	*	*	*	*	-	-	*
Eucalyptus sieberi	*Eucalyptus*	Mytaceae	-	*	-	*	-	-	*
Eucalyptus sclerophylla	*Eucalyptus*	Mytaceae	-	*	-	*	*	*	*

Table 2: Average of leaf relation traits for all four species in sites where there were measured. Data are mean ± SEM shown together with the significance of differences between sites from Tukey's HSD test (p<0.05). Values followed by the same letter for each species are not significantly different. The results are presented for both summer (denoted as S) and winter (denoted as W). Shown in columns are groundwater depth at each site (DGW), osmotic potential at full turgor (π_{100}), leaf water potential at turgor loss point (Ψ_{TLP}), relative water content at turgor loss point (RWC_{TLP}), bulk modulus of elasticity (ε) and specific leaf area (SLA).

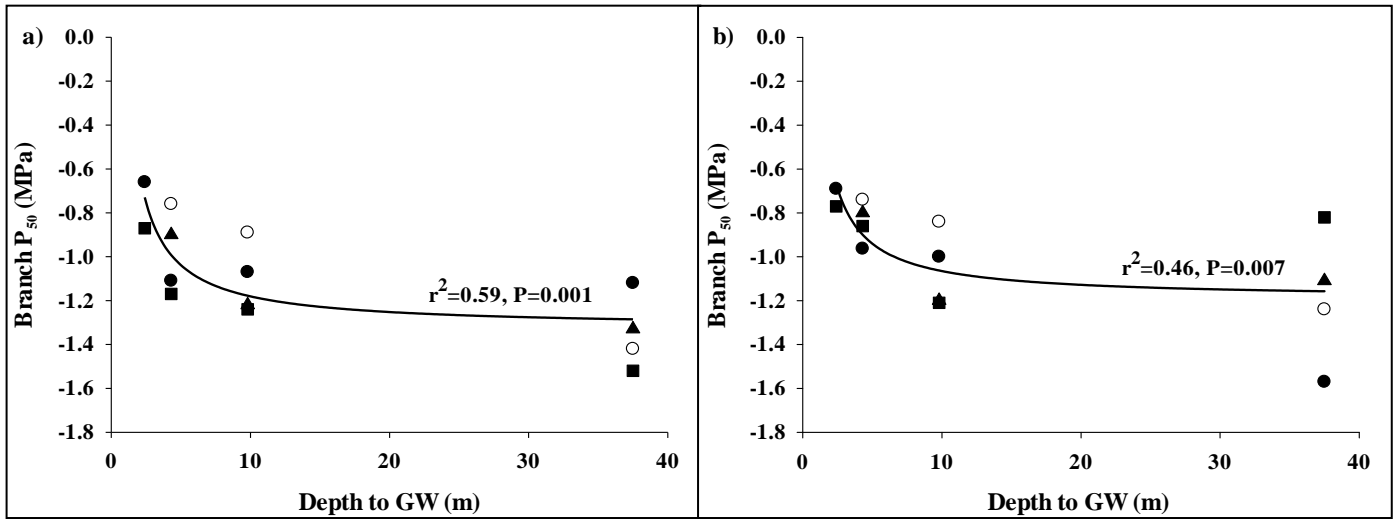| DGW (m)		π_{100} (MPa)	Ψ_{TLP} (MPa)	RWC_{TLP} (%)	ε (MPa)	SLA (cm^2g^{-1})
2.4	W	-0.70±0.04 a	-1.03±0.04 a	94.91±0.21 b	12.20±0.89 a	57.29±1.11 a
	S	-0.67±0.04 a	-0.98±0.05 a	94.12±0.43 ab	10.49±0.82 a	50.55±0.84 ab
4.3	W	-0.74±0.04 a	-1.08±0.04a	95.04±0.26 a	13.66±0.82 ab	51.20±0.61 b
	S	-0.94±0.05 ab	-1.30±0.05b	93.34±0.38 abc	13.14±0.76 ab	47.31±0.54 bc
9.8	W	-1.09±0.06 b	-1.44±0.05 b	93.39±0.41 ab	15.90±1.81 abc	41.92±0.55 c
	S	-1.02±0.04 b	-1.37±0.04 b	92.68±0.31 bc	13.14±0.73 ab	48.08±1.40 abc
13	W	-1.13±0.10 b	-1.52±0.11 b	94.34±0.57 a	18.42±1.18 bc	42.94±1.72 c
	S	-0.92±0.04 ab	-1.28±0.05 b	95.49±0.22 a	19.38±0.82 c	39.51±1.03 d
16.3	W	-1.21±0.13 b	-1.59±0.12 b	94.02±0.57 a	19.36±0.76 c	58.72±2.62 a
	S	-1.09±0.07 b	-1.34±0.06 b	93.03±0.43 bc	14.97±1.22 b	53.37±2.47 a
37.5	W	-1.30±0.06 b	-1.68±0.05 b	91.83±0.30 b	15.17±0.42 ab	49.90±0.62 cd
	S	-1.14±0.05 b	-1.44±0.04 b	91.70±0.47 c	13.92±0.73 ab	44.42±0.80 c

Figure 1: Pooled P_{50} of all species measured at each site as a function of depth-to-groundwater during summer (a) and winter (b). Each symbol repsresents an individual species as following: closed circle (*E. piperita*), closed square (*E. globoidea*), closed triangle (*E. sclerophylla*) and open circle (*E. sieberi*). Curves are exponential decay functions.
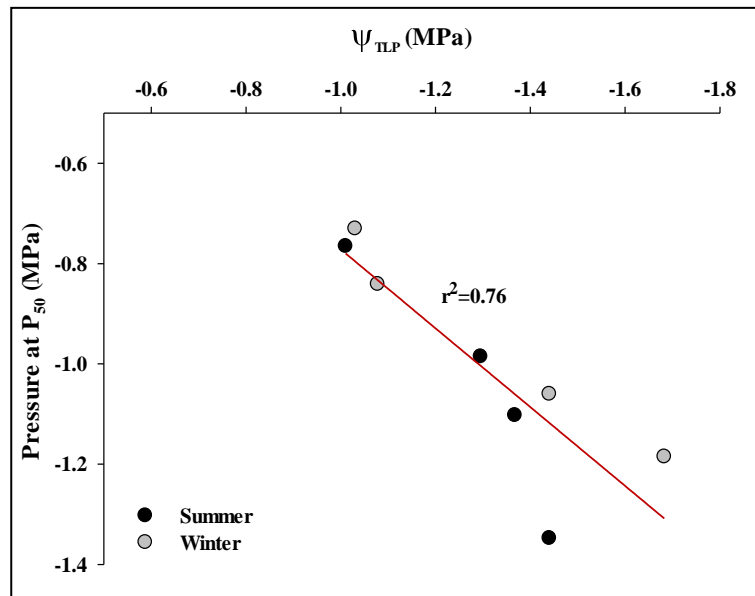
Figure 2: The relationship between leaf water potential at turgor loss point (Ψ_{TLP}) and pressure at which branches lost 50 % of their conductance (P_{50}). Each point is the mean of all species at a single site; winter black circles and summer grey circles.
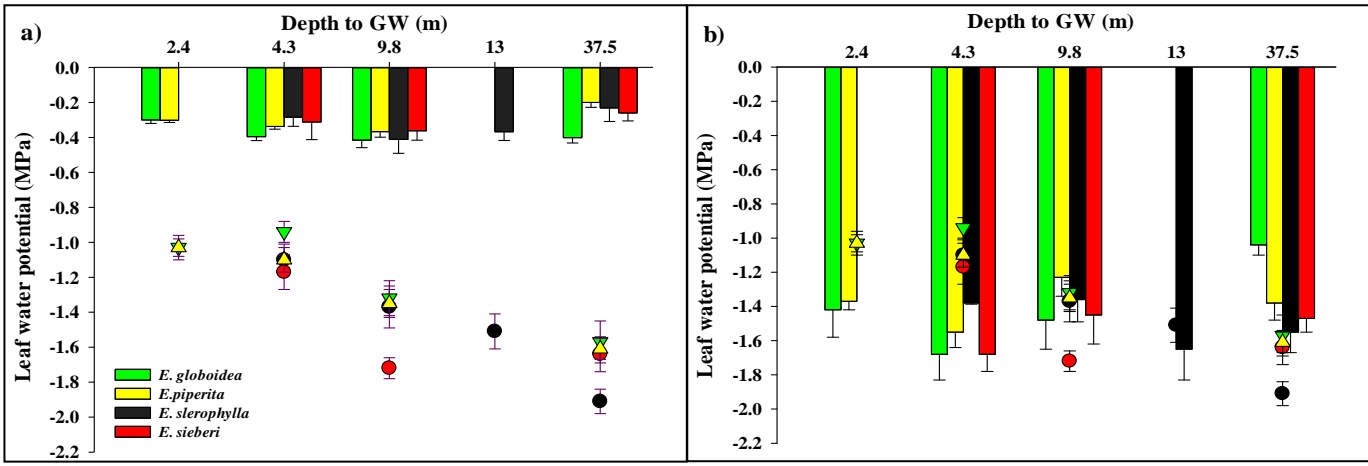
Figure 3: **a)** Pre-dawn leaf water potential (Ψ_{pd}, MPa; bars, n=9) and b) minimum diurnal leaf water potential (Ψ_{min}, MPa; bars, n=9) and leaf water potential at turgor loss point (Ψ_{TLP}, MPa; symbols) during winter for all dominant species growing across a depth-to-groundwater gradient.

The chart shows "Depth to groundwater (m)" on the x-axis (0 to 50) and "Solute potentioal at full turgor (MPa)" on the y-axis (-0.1 to -1.5), with the equation:

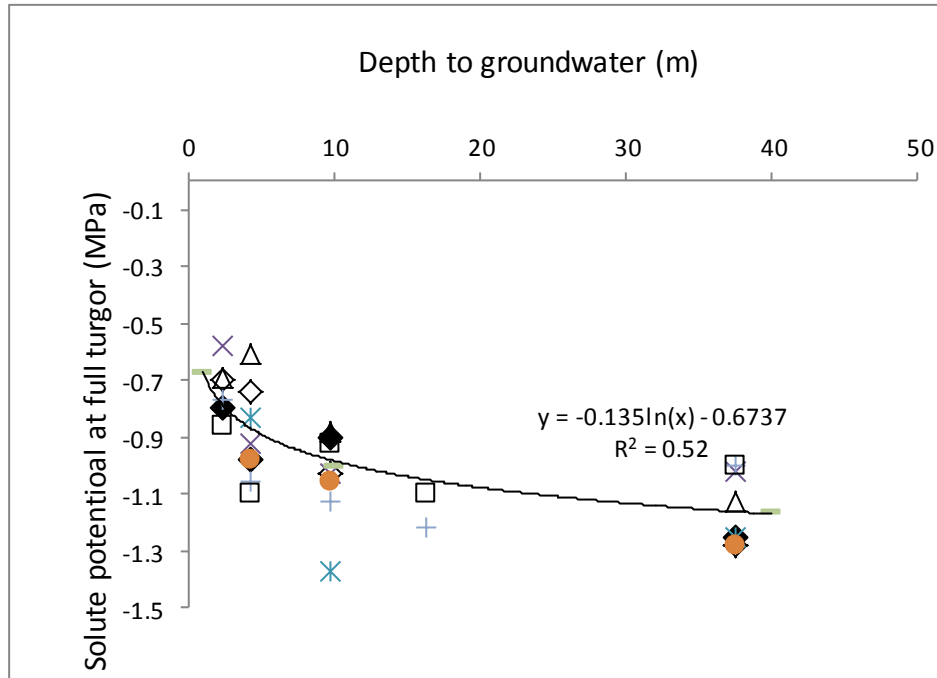$$y = -0.135\ln(x) - 0.6737$$
$$R^2 = 0.52$$

Figure 4:   Changes in solute potential at full turgor for all species as a function of depth to groundwater for winter (closed symbols) and summer (open symbols). Different symbols represent the four species examined (triangle = *E. globoidea*; squares and crosses = *E. sclerophylla*; star = *E. sieberi*; diamonds = *E. piperita*).