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Rapid root elongation by phreatophyte seedlings does not imply tolerance of water table decline

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1 **Rapid root elongation by phreatophyte seedlings does not imply tolerance of water**

2 **table decline.**

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7

8   **Abstract**

9   In a water-limited environment, rapid root elongation immediately after germination can be

10   critical for a plant to reach deeper water sources such as a water table to avoid water deficit

11   stress. However, once plants have accessed a water table their continued survival may

12   depend on their ability to adapt their root distribution to changes in the depth to a water

13   table. In glasshouse experiments using two Banksia species with contrasting water

14   requirements, we investigated i) the rate of root elongation by young seedlings in the

15   presence of a shallow water table, and ii) whole plant response to rapid water table decline

16   using older seedlings that had established root contact with a water table.

17

18   The results of the first experiment agree with the hypothesis that the facultative

19   phreatophyte, *B. attenuata*, has a faster rate of root elongation than the obligate

20   phreatophyte, *B. littoralis*. These differences are likely related to the contrasting habitat

21   preferences of the two species. Older seedlings in the second experiment demonstrated a

22   water saving response to a declining water table, rapidly closing stomata to limit water loss.

23   Additionally, roots did not elongate to follow the water table and plants were quickly

24   disconnected from the saturated zone. For the two phreatophytic *Banksia* species, the

25   capacity for rapid root growth by young seedlings did not translate to an ability for

26   established seedlings to adapt their root distribution to survive rapid water table decline.

27

30   **Key Message:**

[Type text]

31   Despite high rates of root elongation during phreatophyte establishment, once connection to

32   groundwater has occurred and leaf area develops, seedlings demonstrate limited capacity

33   for root elongation in response to groundwater decline.

34   **Introduction**

35   The establishment and persistence of plants, in any given environment, is dependent on the

36   availability of critical resources such as water, nutrients and light (Fenner 1987). Resource

37   requirements are not constant but vary during plant development with some stages, such as

38   recently germinated seedlings, especially susceptible to water stress (Evans and Etherington

39   1991; McDowell et al. 2008). In water limited environments such as Mediterranean-type

40   ecosystems, survival of the first summer drought is critical for plant establishment (Cowling

41   et al. 1987; Frazer and Davis 1988; Richards et al.1995; Padilla and Pugnaire 2007). The

42   presence of a water table within a root zone provides phreatophytic plants (Meinzer 1923)

43   with a source of freely available water (Freeze and Cherry 1979) which is independent of the

44   prevailing rainfall and evaporative conditions. Establishing root contact with the water table

45   as soon as possible minimizes the risk of water deficits and requires traits such as rapid

46   germination and root elongation. However, knowledge about the rate of root elongation is

47   limited due to experimental difficulties associated with measuring this trait despite its

48   importance in determining the success of plants in seasonally water-limited environments.

49

50   The comparative advantage of rapid root elongation is not well understood for

51   phreatophytes. Root responses may differ between obligate phreatophytes which are

52   confined to habitats where plants have permanent access to groundwater, and facultative

53   phreatophytes which utilise groundwater opportunistically (LeMaitre, et al. 1999; Lewis

54   2012). However, the relationship between rates of root elongation and habitat preference is

55   not clearly defined. Slow root elongation has been shown for mesic species that occupy

56   habitats with high water availability across a range of semi–arid ecosystems (Booth et al.

[Type text]

57   1990; Richards et al. 1995; Stave et al. 2005). Conversely, faster root elongation rates have

58   been associated with high rainfall environments when compared across rainfall gradients

59   (Nicotra et al. 2002). For phreatophytic plants, rapid root elongation rates might confer

60   advantages in seasonally arid environments where it is critical to rapidly access deep water

61   reserves as shallow soils become dry (Leishman and Westoby 1994). This trait may facilitate

62   the establishment of facultative phreatophytic species in a wider range of habitats, from

63   shallow to deep depths to water table. In habitats with greater water availability and relatively

64   shallow depths to water table, typically the domain of obligate phreatophytes, rapid root

65   elongation would confer little advantage (Kranjcec et al. 1998).

66

67   In sandy soils, shallow water tables are highly dynamic, rising due to rainfall recharge and

68   falling when the rate of evapotranspiration losses exceed the rate of recharge. Increasingly it

69   is reported that phreatophytic plants have some capacity to adapt to these changes, e.g. the

70   roots of phreatophytic Banksia trees maintained contact with the water table despite a

71   seasonal water table decline of 0.38 cm day^{-1} in sandy soils of south-western Australia

72   (Canham 2011; Canham et al. 2012). Phreatophytes have also been shown to survive

73   artificially rapid rates of water table decline in glasshouse experiments, with plants surviving

74   water table declines of between 1 and 5 cm day^{-1} (Mahoney and Rood 1991; Kranjcec et al.

75   1998; Horton and Clark 2001; Stave et al. 2005; Imada et al. 2010; Gonzalez et al. 2010).

76   Conversely, if the rate of water table decline exceeds the capacity for plants to adjust

77   physiologically, water stress symptoms including reduced photosynthesis and transpiration,

78   branch dieback and eventually plant death, become evident (Scott et al. 1999; Groom et al.

79   2000; Sperry and Hacke 2002; Cooper et al. 2003).

80

81   The impact of water table decline appears to be significant in mature plants which have

82   established in the presence of consistently shallow groundwater (Groom et al. 2000; Froend

      [Type text]

83   and Sommer 2011). Plants growing in mesic conditions can become acclimated to a high

84   level of water availability due to phenotypic plasticity in their physiology (Scott et al. 1999;

85   Maherali et al. 2002; Canham et al. 2009). There may also be a change in water use

86   strategy with age, with seedlings of some species shown to be water spenders in order to

87   establish but subsequently switch to a water saving strategy for long term survival (Richards

88   et al. 1995). Root elongation rates immediately after germination may therefore differ

89   markedly from those of that can be sustained by older plants with large canopies and

90   associated leaf areas.

91

92   In this study we determined the rate of water table decline that two phreatophytic Banksia

93   species (one facultative and one obligate phreatophyte) were able to survive. Two

94   experiments were undertaken; the first was to determine the rate of root elongation for each

95   species during the establishment phase. For this experiment we hypothesised that the

96   species with a distribution restricted to habitats with shallow groundwater would have a

97   slower root elongation rate than the species able survive in deep water table habitats. The

98   second experiment examined the root elongation response of both species to three different

99   rates of water table decline after their roots systems established contact with a consistent

100   water table. We predicted that the response of these older plants to a decline in water table

101   would be to maintain root contact with groundwater at elongation rates equivalent to those

102   observed in the first experiment.

103

104   **Materials and Methods**

105   Two congeneric species of *Banksia* (Proteaceae) from the Mediterranean-type environment

106   of south-west Australia with contrasting water requirements were studied; *Banksia attenuata*

107   R.Br., a facultative phreatophyte with a wide distribution relative to depth to water table

[Type text]

108   (Zencich et al. 2002), and *Banksia littoralis* R.Br. an obligate phreatophyte, confined to

109   damplands with shallow depth to groundwater (Arrowsmith 1992; Zencich et al. 2002).

110

111   *Rhizopod Design*

112   Experiments were undertaken using seedlings grown in rhizopods (*sensu* Mahoney and

113   Rood 1991) in a glasshouse. In experiment 1 we constructed 24 rhizopods using two 2 m tall

114   tubes of 90 mm diameter PVC attached to a reservoir to maintain and manipulate water

115   table depths (n=48 tubes). Tubes were filled with a medium to coarse sand representative of

116   the soil in their natural habitats. The soil had low water holding capacity, with wilting point (-

117   1.5 MPa) and field capacity occurring at 0.04 % and 0.07 % respectively. Each tube

118   contained 1 plant and each species was represented within each rhizopod (i.e. 24 replicates

119   of each species). An additional 5 rhizopods (5 replicate plants per species) were

120   constructed, the tubes of which had a clear polycarbonate wall (covered with black plastic to

121   keep dark) for non-destructive observation of root growth. In experiment 2, 21 rhizopods

122   were used of a similar configuration to experiment 1, giving 21 replicate plants for each of

123   the 2 species.

124

125   *Experiment 1: Rate of root elongation*

126   To test the first hypothesis seedlings were germinated and transplanted into the rhizopod

127   tubes in March, 8 weeks after sowing when seedlings still had green cotyledons and a

128   maximum of 4 true leaves. The soil was prepared by adding slow release fertiliser (18 g of

129   Osmocote[©] Native Plants) to the top 5 cm of each tube. Seedlings were watered every 3

130   days after transplanting for a period of 33 days after which the water table was the only

131   water source available. The temperature of the glasshouse ranged from 18 ºC to 25 ºC and

[Type text]

132 relative humidity ranged between 25 % and 90 % averaging 50 % across the experimental

133 period.

134

135 Seedlings were sampled on the 33rd, 49th, 65th and 92nd day after transplanting with 6

136 replicates per species per sampling period. Plants were washed free of sand and intact total

137 root and shoot lengths were recorded. Shoots and root portions cut into 20 cm increments

138 were dried at 40 ºC until reaching a constant weight. Whole seed weight was determined by

139 weighing 20 individual seeds of each species. Total leaf area was measured prior to drying

140 using a leaf area meter (model Delta-T Type WDIGC-2, Delta T Devices, Cambridge UK.)

141 and leaf water potential (*n* = 3) was determined using a dewpoint potentiometer. Volumetric

142 soil water content and soil water potential (using a dewpoint potentiometer; Decagon

143 Services WP4) were measured at 5 cm increments down the soil profile at each sampling

144 period.

145

146 Root elongation rates were determined for destructively sampled seedlings by determining

147 the change in root length from the previous sample period, represented as:

$$RER = \frac{x_i - \bar{x}_{i-1}}{n_i - n_{i-1}}$$

148 Where RER = root elongation rate, n = number of days, x = sample root length, $\bar{x}$ = mean

149 root length.

150 Root elongation rates for the seedlings in the window tubes were calculated by determining

151 the difference in root length since the previous observation and then dividing by the number

152 of days between observations (*n* = 5). The capillary fringe was determined to be 20 cm

153 above the water table and 80 cm below the soil surface. Window observations of roots were

154 used to determine the number of days it took for each individual to reach 80 cm depth.

[Type text]

155

Plant response to water table decline

Seedlings were transplanted into rhizopods tubes using the same procedure as the first experiment and were left to establish with a water table 1 m below the soil surface and no surface watering after the first 8 weeks. Three different rates of water table decline were used to investigate root response of the study species. Based on the maximum root elongation rates observed in the first experiment the water table decline rates were as follows; 2 cm day^{-1} (less than or equivalent to maximum root elongation rates of both species), 4 cm day^{-1} (equivalent to maximum rate of *B. attenuata* but exceeding rate of *B. littoralis*) and 10 cm day^{-1} (exceeds maximum rate of both species). Roots did not extend into the saturated zone in the first experiment but were limited to the unsaturated zone and capillary fringe, therefore a control of 0 cm day^{-1} rate was not used as it was assumed root elongation would not occur beyond the water table. There were seven replicates of each species in each treatment for plant harvesting at the end of the experiment and three replicates per species per treatment for soil moisture measurement.

170

The commencement of treatments was staggered so that all finished within a similar period of each other to limit differences in plant age at the end of the experiment. The water table started at 100 cm from the soil surface and was drawn down by 80 cm to 180 cm below the soil surface in all treatments. Water table levels were lowered using a tap at the bottom of the reservoir and the rate of decline was regulated in a step-wise manner on a daily basis. The 2 cm day^{-1} treatment started 16 weeks after transplanting and the water table decline took 40 days to reach the 180 cm level. The 4 cm day^{-1} treatment started 17 weeks after transplanting and lasted 20 days, and the 10 cm day^{-1} treatment started 20 weeks after transplanting and took eight days. Whilst it was recognised that this staggered start to treatments would result in different starting ages of seedlings, our focus was on comparing

[Type text]

181 species responses to the treatments. Once the lowering of the water table was completed

182 the seedlings were left in the rhizopods for a further 30 days to allow plants to adjust to the

183 new water table depth. Glasshouse temperatures were controlled and ranged between 18°C

184 and 25°C with an average relative humidity of 50%.

185

186 To investigate plant response to the declining water table, transpiration, stomatal

187 conductance and photosynthesis rates were measured within a two hour period during the

188 middle of the day using an infra-red gas analyser (IRGA; Model Li 6400, Li-cor Inc., Lincoln

189 Nebraska). Measurements were made at time 0 (i.e. the day that water table decline

190 commenced) then every 10 days until plants were harvested. For each plant, gas exchange

191 was measured on the youngest fully expanded leaf. Prior to harvesting, predawn plant water

192 potentials of the whole plant were determined using a Scholander-type pressure chamber

193 (Model 3005, PMS instruments, Oregon, USA) as a measure of hydraulic connection to the

194 capillary fringe. Leaf area was determined using an area meter (model Delta-T Type

195 WDIGC-2, Delta T Devices, Cambridge UK) before drying leaves and shoots. Roots were

196 sampled in 20 cm increments, washed free from sand, and then dried and weighed.

197 Volumetric soil water content was determined at 5 cm increments on three occasions for

198 each treatment; the day when the water table decline treatments began, when the treatment

199 ceased and when plants were harvested. Additionally, the water content in millilitres was

200 determined for the top 100 cm of the soil profile.

201

202 *Data Analysis*

203 To test for differences between study species in the root elongation rate experiment,

204 independent t-tests were performed on each parameter (root elongation rate, root mass,

205 shoot mass, root:shoot ratio, seed weight and pre-dawn plant water potential) at each

206 sampling period. Where data did not meet normality requirements a log transformation was

[Type text]

207 used. Leaf area and root:shoot ratio data could not be transformed to a normal distribution

208 so non-parametric Kruskal-Wallis tests were used to test for significant differences.

209

210 For the response to water table decline experiment, total root and shoot dry weights were

211 compared between each species and treatment using 2-way analysis of variance (ANOVA)

212 and a Tukey *post hoc* test was applied where differences were detected. Leaf area,

213 root:shoot ratios and predawn shoot water potentials could not be transformed to meet the

214 assumptions required for parametric statistical testing, and were therefore analysed using

215 the non-parametric Kruskal-Wallis test. Additionally, the relationship between leaf area and

216 predawn xylem pressure potential was examined using a Spearman's rank order correlation.

217 All analyses were carried out using IBM SPSS version 19.0 software (SPSS Inc., Chicago,

218 IL, USA).

219

220 **Results**

221 *Experiment 1: Rate of root elongation*

222 Soil moisture in the unsaturated zone was initially high, ranging between 1 and 5%

223 throughout the soil profile (Fig. 1). However, after hand watering at the soil surface ceased

224 the soil profile dried progressively from the surface down so that by the conclusion of the

225 experiment, 75% of the unsaturated profile had less than 1% volumetric water content in

226 tubes containing *B. attenuata*. However, in *B. littoralis* tube soil moisture at the end of the

227 experiment was higher with only 40% of the soil profile having less than 1% volumetric water

228 content. Although a significant portion of the unsaturated profile dried during the course of

229 the experiment the water table at 1 m (and the capillary fringe extending 20 cm above it)

230 provided a constant water source for the duration of the experiment.

231

[Type text]

232   Seedlings of both study species extended their roots to the capillary fringe by the end of the

233   experiment (92 days after transplanting; Fig. 1) and therefore the total root length attained

234   was not significantly different between species (101.8 ± 3 cm for *B. littoralis* and 95.0 ± 4 cm

235   for *B. attenuata*). Peak elongation rates were observed 8 days after hand watering had

236   stopped, with the facultative phreatophyte *B. attenuata* having a faster rate of root elongation

237   than the obligate phreatophyte, *B. littoralis*. (Fig. 2a and b). This resulted in the *B. attenuata*

238   roots reaching the capillary fringe by day 46 ± 4 whilst the obligate phreatophyte *B. littoralis*

239   took significantly longer at 69 ± 5 days (t (8) = -4.437, p < 0.01).

240

241   The seeds of *Banksia attenuata* had a greater mass (mean weight of 108 ± 4mg) relative to

242   *B. littoralis* seeds (21 ± 1 mg; Kruskal-Walis, $\chi^2(40)$ = 29.268, p < 0.001). By the final

243   sampling period *B. attenuata* had greater root (t (10) = 4.298, p < 0.01; Table 1) and shoot (t

244   (10) = 5.423, p < 0.001) mass and twice the leaf area of *B. littoralis* (Kruskal-Wallis, χ^2 (12) =

245   5.769, p < 0.01). Cotyledon leaves were larger for *B. attenuata* and remained green for

246   longer (Table 2). By the end of the experiment, the majority of *B. littoralis* seedlings (87%)

247   had brown, shrivelled cotyledon leaves whereas in *B. attenuata* 33% still had green

248   cotyledon leaves. There were no significant interspecific differences in leaf water potential

249   and values were consistent at each sampling period although it was highest at day 33 before

250   the upper parts of the soil profile started to dry.

251

252   *Experiment 2: Plant response to water table decline*

253   The sandy soil had limited water-holding capacity, with water in the capillary fringe and

254   saturated zone draining rapidly as water table decline occurred (Fig. 3). At the end of the 2nd

255   experiment, the 2 cm day^{-1} treatment had similar volumetric water content in the soil profile

256   as the 4 cm day^{-1} and 10 cm day^{-1} treatments. This is despite the 2 cm day^{-1} decline taking

257   40 days to complete, while the 10 cm day^{-1} treatment took just eight days. Additionally, there

     [Type text]

258 was little change in the soil water content between the end of the water table decline

259 treatment and 30 days after the treatment ended.

260

261 There was only limited evidence of a root elongation response following a declining water

262 table in both species, with no roots observed below 120 cm from the soil surface at the end

263 of the experiment. For *B. attenuata*, there did not appear to be any root elongation response

264 to water table decline, with roots remaining in the 80 to 100 cm portion of the soil profile

265 (position of the capillary fringe prior to water table decline) for all rate treatments (Fig. 3). In

266 contrast, the obligate phreatophyte *B. littoralis*, showed a limited response to water table

267 decline in all three treatments with roots extended beyond the pre-decline capillary zone to

268 120 cm from the surface.

269

270 As water content in the top 1 m of the soil profile (the unsaturated vadose zone above the

271 starting water table level) decreased over the course of the experiment, photosynthetic rates

272 and stomatal conductance decreased for both species (Fig. 4). By 30 days after the start of

273 decline all treatments and both species appear to have been disconnected from the water

274 table/capillary fringe with very low rates of photosynthesis observed. Photosynthesis rates

275 dropped rapidly for both species, particularly in the 4 and 10 cm day^{-1} treatments, taking 10

276 days to reach less than 6 µmol m^{-2} s^{-1}. However, it took 30 days to reach a similar value in

277 the 2 cm day^{-1} treatment and corresponds with the longer duration of higher soil water

278 storage in this treatment. Stomatal conductance followed a similar trend with the rate of

279 decline greatest in the 10 cm day^{-1} water table decline treatment and a significantly slower

280 response for the 2 cm day^{-1} treatment.

281

[Type text]

282   The facultative phreatophyte, *B. attenuata*, had significantly larger leaf area (Kruskal-Wallis

283    $\chi^2(32) = 21.05$, $p < 0.001$) and greater shoot mass (ANOVA $f(32)=34.51$, $p < 0.001$) than the

284   obligate phreatophyte *B. littoralis* across all treatments (Table 3). Additionally, predawn

285   xylem pressure potentials were positively correlated with plant leaf area ($r= 0.772$, $p < 0.001$,

286    $n=31$) with larger leaf area seedlings tending to be more water deficit stressed by the end of

287   the experiment. Higher seedling root and shoot biomass in the 10 cm day^{-1} treatment (Table

288   3) is considered a consequence of the greater age of seedlings at the start of treatment (due

289   to the staggered start). Differences and similarities between species however were

290   consistent irrespective of treatment rate.

291

Discussion

293   Our hypothesis that the facultative phreatophyte *B. attenuata* has a faster rate of root

294   elongation at the seedling establishment phase than the obligate phreatophyte *B. littoralis,* is

295   supported by the first experiment. The difference in the rate of root elongation between these

296   species may reflect their habitat preferences, e.g. *B. littoralis* confined to habitats that afford

297   year-round access to a comparatively shallow water table (Zencich et al. 2002). In contrast

298   *Banksia attenuata* is distributed across a range of hydrological habitats including xeric

299   environments where there is no access to a water table. In these habitats rapid root

300   elongation deep into the soil profile may be advantageous for seedlings growing on course

301   sandy soils in a Mediterranean-type climate, allowing plants to rapidly exploit deeper soil

302   moisture stores during long, dry summers. The greater seed mass of *B. attenuata* also

303   supports this hypothesis and facilitates higher rates of root growth early in the seedling

304   establishment phase.

305

306   We predicted that once established, seedlings of the study species would maintain contact

307   with a water table declining at rates similar to the rates of root elongation observed in the

[Type text]

308   first experiment; however the study species demonstrated a weak root elongation response

309   to all three rates of water table decline. The notion that the roots of phreatophytic plants are

310   able to extend at such a rate is supported in the literature. Stave et al. (2005) found that

311   *Faidherbia albida* seedlings survived a water table declining at a rate of 5 cm day^{-1}. Similarly,

312   Kranjcec et al. (1998) reported that *Populus balsamifera* maintains contact with a water table

313   declining at 4 cm day^{-1}. The same study also indicated a root growth response following a 10

314   cm day^{-1} rate of decline although plant water relations data indicate that this rate was too

315   fast for roots to maintain contact with the water table. Plants in the current study showed

316   similar signs of water stress which was associated with seedlings disconnecting from the

317   water table through lack of root elongation. Having established contact with a consistent

318   water table and increased shoot biomass and leaf area, seedlings in the second experiment

319   were unable to respond to a water table decline at a rate equivalent to the root elongation

320   rate observed prior to establishing contact with the water table.

321

322   The initial rate of root elongation by recently germinated seedlings differs to the rate

323   observed for established seedlings (i.e. root contact with water table) responding to a decline

324   in water table. Initial growth by recently germinated seedlings is linked to seed size in many

325   species, with large seeds producing larger seedlings than small seeds (Marshall 1986;

326   Jurado and Westoby 1992; Osunkoya et al.1993; Leishman and Westoby 1994). In

327   particular, cotyledon reserves of carbon, nitrogen and phosphorus are critical for the early

328   vegetative growth of Banksia seedlings in water-limited and nutrient-poor environments

329   (Groom et al. 2001; Lamont and Groom 2013). In this study it was found that *Banksia*

330   *attenuata* has seeds five times larger and seedlings (3 months old) twice as large as *B.*

331   *littoralis*. The difference in seed and seedling size between the study species is reflected in

332   the post-emergence root elongation rates with the facultative phreatophyte elongating roots

333   twice as fast as the obligate phreatophyte. However, once seed reserves are exhausted

334   large seededness may no longer influence seedling growth rates, impacting on the ability for

[Type text]

335 plants to respond to altered environmental conditions such as a decline in the water table

336 (Leishman et al. 2000; Walters and Reich 2000; Dalling and Hubbell 2002). The rapid root

337 elongation rates by young seedlings in the first experiment may be a result of an initial race

338 to develop extensive root systems fuelled by cotyledon reserves. However, the comparative

339 advantage of large seeds did not benefit older seedlings of *B. attenuata* which did not

340 elongate their roots to follow the declining water table.

341

342 For the ongoing recruitment and survival of phreatophytes it is important to consider the

343 physiological differences that occur in the lifecycle of the plants. Rapid root elongation by

344 seedlings soon after germination is critical for plants to connect with the water table

345 particularly in water-limited environments. Once their roots are in contact with the water table

346 or capillary fringe above it, phreatophytic plants are likely to adjust their water requirements

347 according to water availability, for example by increasing leaf area (Eagleson 2002). These

348 established plants are able to adapt to some changes in the water table level, for example

349 by adjusting root length in response to the seasonal recharge and decline of an unconfined

350 aquifer (Canham et al. 2012). However, if the magnitude and rate of change is great, plants

351 may become disconnected from the water table if their rate of root elongation is insufficient

352 to maintain contact. High transpirational demand increases the tension of the hydraulic

353 continuum making it susceptible to embolism. The hydraulic gradient is greatest at the root-

354 soil interface and once embolisms occur in the roots, there is a significant decrease in the

355 amount of water able to be transported through the plant resulting in hydraulic failure and

356 plant death (Sparks & Black 1999; McDowell et al. 2008). Thus, the large leaf area afforded

357 by a greater water availability such as a shallow water table, may increase the susceptibility

358 of older plants to rapid decline in the water table.

359

[Type text]

360 Like many phreatophytic species globally the Banksia study species are being impacted by

361 changes to the hydrological cycle (Naumburg et al. 2005). In the case of south-western

362 Australia declining water tables continue to threaten populations of phreatophytic species

363 and changes in vegetation composition have already been demonstrated (Froend and

364 Sommer 2011). The recruitment of obligate phreatophytic species, such as *B. littoralis* may

365 well depend on the ability of seedlings to develop root systems to access shallow water

366 tables. With progressive increases in depth to groundwater access to this important water

367 source before the onset of the summer dry period may not occur, increasing the risk of water

368 deficits.

369

370 In contrast to obligate phreatophytes, facultative phreatophytic species are able to rapidly

371 establish a deep root system to access subsoil moisture and groundwater. *B. attenuata*

372 rapidly develops a deep root system within the first month of seedling growth and therefore

373 can successfully establish in wider range of habitats, including areas with no access to a

374 water table (Zencich et al. 2002). Populations of *B. attenuata* are therefore likely to continue

375 to recruit, relative to *B. littoralis* despite long-term changes in depth to water table. However,

376 our data suggest individuals of *B. attenuata* that have established in habitats with a shallow,

377 consistent water table are vulnerable to rapid drops in the depth of the water table. Although

378 limited to observations made on seedlings, the results of our second experiment suggest

379 mesic antecedent conditions allow these plants to develop large leaf areas and therefore

380 greater transpirational demand. Additionally, there can be intraspecific plasticity in plant

381 hydraulic architecture such as increased vulnerability to xylem embolism associated with

382 populations that have established in wetter habitats (Canham et al. 2009). In combination,

383 these attributes are likely to contribute to increased risk of hydraulic failure in response to a

384 rapidly declining water table, inhibiting the plant's capacity for root elongation and resulting in

385 the disconnection from the water table and plant death.

[Type text]

386

Acknowledgements

Conflict of Interest

395

[Type text]

396

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Tables

Table 1. Summary of plant parameters measured on destructively sampled seedlings for experiment 1. All data are mean ±SE (n = 6, except for Ψ leaf where n = 3). * indicates a significant difference detected between *Banksia attenuata* (BA) and *Banksia littoralis* (BL) at that sampling period as determined using t tests and † indicates a significant difference detected using Kruskal-Wallis test, as data did not meet the normality of distribution requirements for parametric analysis.

Parameter	Spp.	Day 33	Day 92
Root Weight (mg)	BA	50.5 ± 7.4*	600.4 ± 22.9*
	BL	91.3 ± 17.2*	231.1 ± 39.7*
Shoot Weight (mg)	BA	168.0 ± 19.2	866.7 ± 72.3*
	BL	156.7 ± 26.8	360.0 ± 59.2*
Leaf Area (cm^2)	BA	9.5 ± 2.2	54.0 ± 5.7†
	BL	13.7 ± 1.9	25.6 ± 5.6†
Pre-dawn Ψ_{leaf} (MPa)	BA	-0.6 ± 0.1	-1.2 ± 0.0
	BL	-0.8 ± 0.1	-1.4 ± 0.4

Table 2. Colour of cotyledons observed on harvesting days 33 and 92. Values are a percentage of total plant numbers for *Banksia attenuata* (BA) and *Banksia littoralis* (BL) and n = 6 for each species.

		Cotyledon Colour		
		Green	Yellow	Brown
Day 33	BA	100	0	0
	BL	100	0	0
Day 92	BA	33	67	0
	BL	0	17	83

[Type text]

Table 3. Summary of plant parameters for experiment 2, investigating plant response to declining water tables at rates of 2, 4 and 10 cm per day^{-1}. ^{A}, ^{AB} and ^{B} indicate significant differences between treatments for total root weight, ^{a} and ^{b} indicate significant differences between study species (2-way ANOVA with Tukey post-hoc test, sig. <0.05). ^{c} and ^{d} indicate significant differences in root:shoot ratio, leaf area and predawn shoot water potential (Ψ_{PD}) between species (Kruskal-Wallis, sig. <0.05).

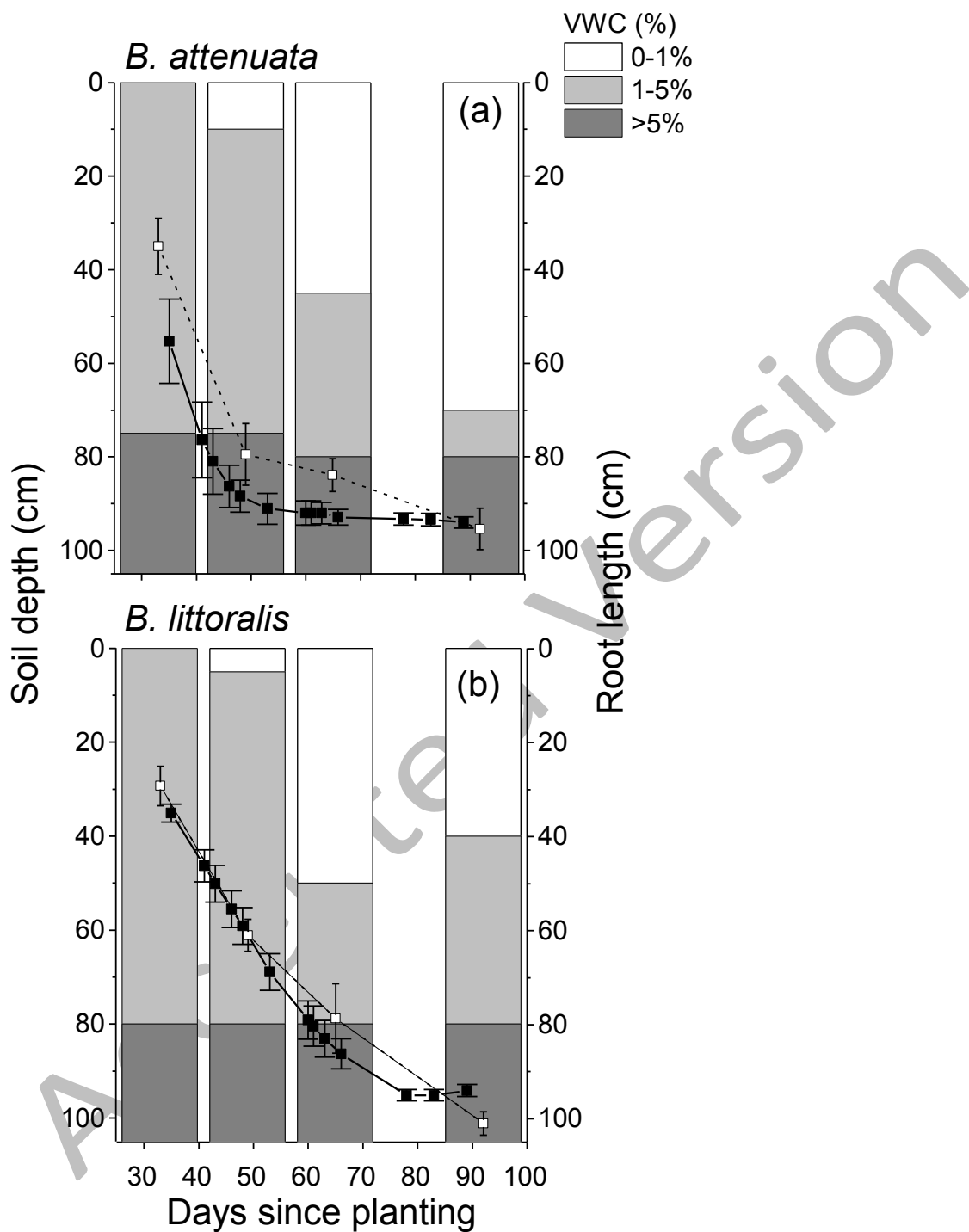| Parameter	Spp.	2 cm	4 cm	10 cm
Total Root Weight (g)	BA	1.6 ± 0.3^A	2.0 ± 0.3^{AB}	2.7 ± 0.5^B
	BL	1.0 ± 0.2^A	1.4 ± 0.4^{AB}	2.4 ± 0.5^B
Total Shoot Weight (g)	BA	2.8 ± 0.6^a	3.1 ± 0.6^a	4.3 ± 0.6^a
	BL	0.7 ± 0.1^b	1.0 ± 0.3^b	1.3 ± 0.4^b
Root:Shoot (-)	BA	0.6 ± 0.0^c	0.8 ± 0.2^c	0.6 ± 0.1^c
	BL	1.7 ± 0.1^d	1.5 ± 0.1^d	2.0 ± 0.3^d
Leaf Area (cm^2)	BA	145.5 ± 29.0^c	149.4 ± 32.8^c	189.2 ± 22.5^c
	BL	47.3 ± 12.3^d	62.6 ± 19.0^d	97.8 ± 30.7^d
Ψ_{PD} (MPa)	BA	-2.4 ± 0.6^c	-2.8 ± 0.4^c	-2.2 ± 0.4^c
	BL	-0.9 ± 0.4^d	-1.3 ± 0.6^d	-1.4 ± 0.5^d

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Figure 1. Volumetric water content (VWC; n = 1 for both species) and root lengths recorded through observation windows (closed symbols) and destructive sampling (open symbols) for *Banksia attenuata* (top) and *Banksia littoralis* (bottom). Volumetric water content is categorised as 0-1%, 1 to 5% and more than 5% VWC. Root length values are mean ± 1 SE (shaded area) and n = 5.

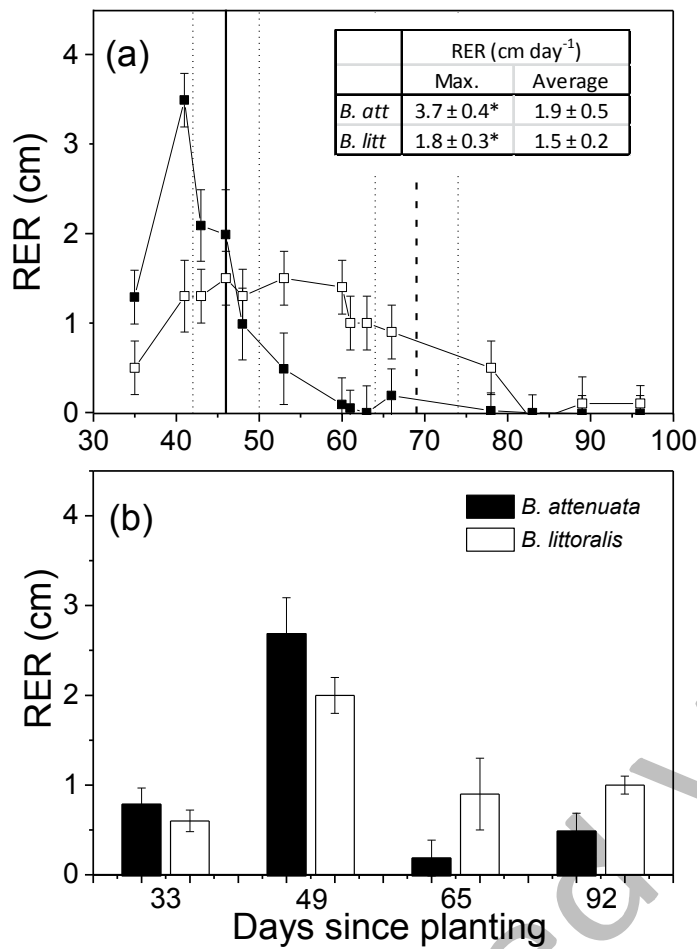[Type text]

Figure 2. Root elongation rates for *Banksia attenuata* (solid symbols and columns) and *Banksia littoralis* (open symbols and columns) as determined by (a) observation windows and by (b) destructive sampling. Vertical lines in (a) represent the day that roots reached the capillary fringe (80 cm) for *B. attenuata* (solid line) and *B. littoralis* (dashed line; dotted line is standard error for each species), which was significantly different (t(8)=-4.437, p <0.05). Insert shows mean and standard errors for root elongation rates (n = 5) of *Banksia attenuata* and *Banksia littoralis* as observed through root windows. Max. refers to the fastest root elongation rate observed for each individual; Average is the mean RER between cessation of watering (33 days) and when the roots reached the capillary fringe. * indicates a significant difference between species (t tests, p <0.05).
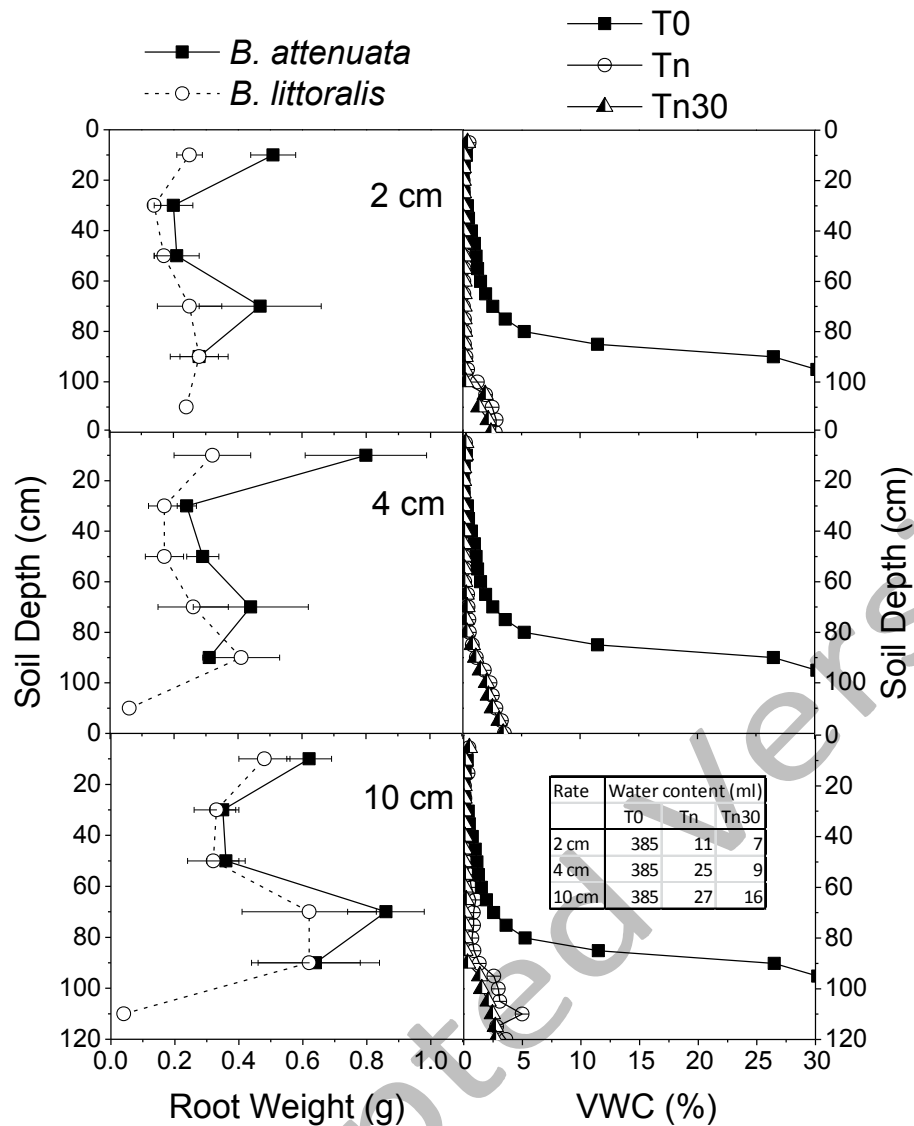
Figure 3. Root dry weights (left) at 20 cm increments for *Banksia attenuata* (n= 4 for 2 cm day^{-1} and 4 cm day^{-1} treatments, and 7 for 10 cm day^{-1}), and *Banksia littoralis* (n = 5 for 2 cm day^{-1} treatment and 6 for the 4 cm day^{-1} and 10 cm day^{-1}) harvested 30 days after 2 cm day^{-1} 4 cm day^{-1} and 10 cm day^{-1} water table decline treatments. Volumetric water content (VWC) (right) before decline start (T0), at decline end (Tn) and 30 days after decline finished (Tn30; n=2). Insert shows the water content (ml) in the top 100 cm of the soil profile at the beginning, end and 30 days after the water table decline treatments.
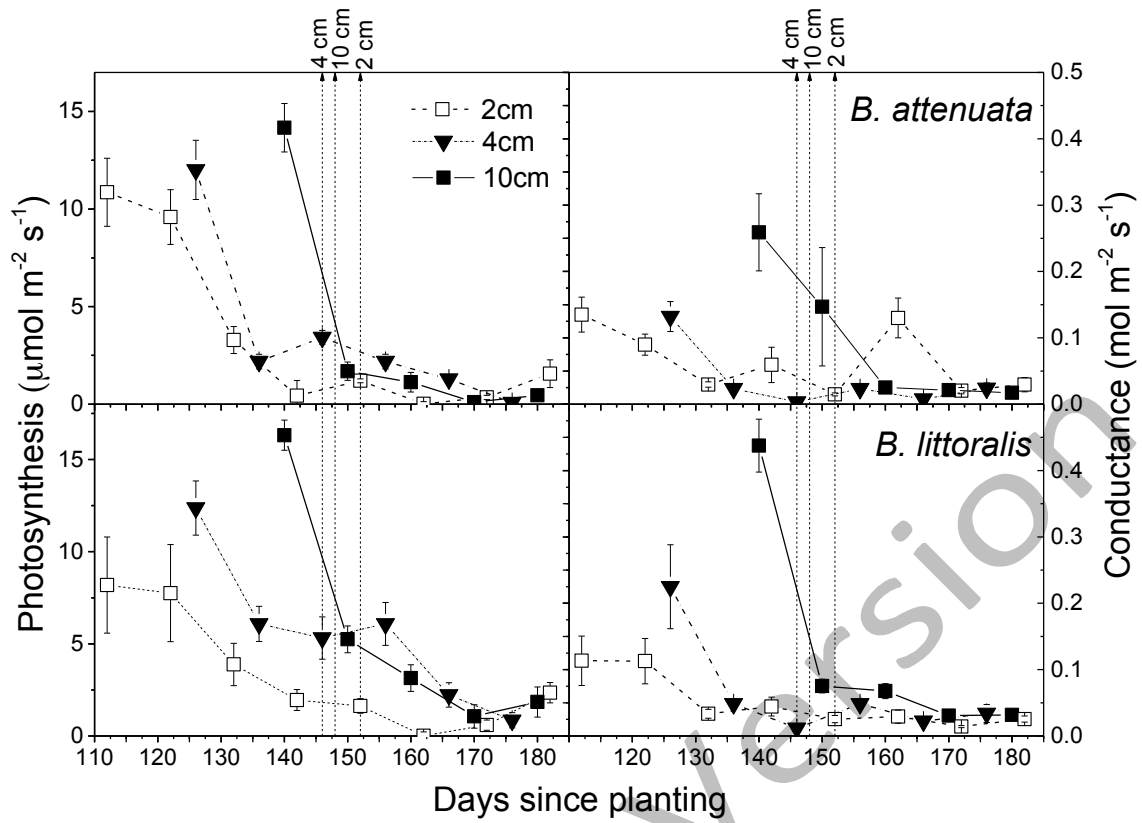
Figure 4. Rate of photosynthesis and stomatal conductance (mean +/-1 SE) of *B. attenuata* and *B. littoralis* (n = 7) seedlings exposed to water table decline of 2 cm, 4 cm and 10 cm per day. The water table drawdown treatment began the day after initial gas exchange measurements. Dashed vertical lines represent the end of the water table drawdown treatment.