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

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Abstract

In a water-limited environment, rapid root elongation immediately after germination can be critical for a plant to reach deeper water sources such as a water table to avoid water deficit stress. However, once plants have accessed a water table their continued survival may depend on their ability to adapt their root distribution to changes in the depth to a water table. In glasshouse experiments using two *Banksia* species with contrasting water requirements, we investigated i) the rate of root elongation by young seedlings in the presence of a shallow water table, and ii) whole plant response to rapid water table decline using older seedlings that had established root contact with a water table.

The results of the first experiment agree with the hypothesis that the facultative phreatophyte, *B. attenuata*, has a faster rate of root elongation than the obligate phreatophyte, *B. littoralis*. These differences are likely related to the contrasting habitat preferences of the two species. Older seedlings in the second experiment demonstrated a water saving response to a declining water table, rapidly closing stomata to limit water loss. Additionally, roots did not elongate to follow the water table and plants were quickly disconnected from the saturated zone. For the two phreatophytic *Banksia* species, the capacity for rapid root growth by young seedlings did not translate to an ability for established seedlings to adapt their root distribution to survive rapid water table decline.

Keywords: Proteaceae, Mediterranean-type ecosystem, rhizopod, *Banksia*, *phreatophyte*

Key Message:

Despite high rates of root elongation during phreatophyte establishment, once connection to groundwater has occurred and leaf area develops, seedlings demonstrate limited capacity for root elongation in response to groundwater decline.

Introduction

The establishment and persistence of plants, in any given environment, is dependent on the availability of critical resources such as water, nutrients and light (Fenner 1987). Resource requirements are not constant but vary during plant development with some stages, such as recently germinated seedlings, especially susceptible to water stress (Evans and Etherington 1991; McDowell et al. 2008). In water limited environments such as Mediterranean-type ecosystems, survival of the first summer drought is critical for plant establishment (Cowling et al. 1987; Frazer and Davis 1988; Richards et al. 1995; Padilla and Pugnaire 2007). The presence of a water table within a root zone provides phreatophytic plants (Meinzer 1923) with a source of freely available water (Freeze and Cherry 1979) which is independent of the prevailing rainfall and evaporative conditions. Establishing root contact with the water table as soon as possible minimizes the risk of water deficits and requires traits such as rapid germination and root elongation. However, knowledge about the rate of root elongation is limited due to experimental difficulties associated with measuring this trait despite its importance in determining the success of plants in seasonally water-limited environments.

The comparative advantage of rapid root elongation is not well understood for phreatophytes. Root responses may differ between obligate phreatophytes which are confined to habitats where plants have permanent access to groundwater, and facultative phreatophytes which utilise groundwater opportunistically (LeMaitre, et al. 1999; Lewis 2012). However, the relationship between rates of root elongation and habitat preference is not clearly defined. Slow root elongation has been shown for mesic species that occupy habitats with high water availability across a range of semi-arid ecosystems (Booth et al.

1990; Richards et al. 1995; Stave et al. 2005). Conversely, faster root elongation rates have been associated with high rainfall environments when compared across rainfall gradients (Nicotra et al. 2002). For phreatophytic plants, rapid root elongation rates might confer advantages in seasonally arid environments where it is critical to rapidly access deep water reserves as shallow soils become dry (Leishman and Westoby 1994). This trait may facilitate the establishment of facultative phreatophytic species in a wider range of habitats, from shallow to deep depths to water table. In habitats with greater water availability and relatively shallow depths to water table, typically the domain of obligate phreatophytes, rapid root elongation would confer little advantage (Kranjcec et al. 1998).

In sandy soils, shallow water tables are highly dynamic, rising due to rainfall recharge and falling when the rate of evapotranspiration losses exceed the rate of recharge. Increasingly it is reported that phreatophytic plants have some capacity to adapt to these changes, e.g. the roots of phreatophytic Banksia trees maintained contact with the water table despite a seasonal water table decline of 0.38 cm day⁻¹ in sandy soils of south-western Australia (Canham 2011; Canham et al. 2012). Phreatophytes have also been shown to survive artificially rapid rates of water table decline in glasshouse experiments, with plants surviving water table declines of between 1 and 5 cm day⁻¹ (Mahoney and Rood 1991; Kranjcec et al. 1998; Horton and Clark 2001; Stave et al. 2005; Imada et al. 2010; Gonzalez et al. 2010). Conversely, if the rate of water table decline exceeds the capacity for plants to adjust physiologically, water stress symptoms including reduced photosynthesis and transpiration, branch dieback and eventually plant death, become evident (Scott et al. 1999; Groom et al. 2000; Sperry and Hacke 2002; Cooper et al. 2003).

The impact of water table decline appears to be significant in mature plants which have established in the presence of consistently shallow groundwater (Groom et al. 2000; Froend

and Sommer 2011). Plants growing in mesic conditions can become acclimated to a high level of water availability due to phenotypic plasticity in their physiology (Scott et al. 1999; Maherali et al. 2002; Canham et al. 2009). There may also be a change in water use strategy with age, with seedlings of some species shown to be water spenders in order to establish but subsequently switch to a water saving strategy for long term survival (Richards et al. 1995). Root elongation rates immediately after germination may therefore differ markedly from those of that can be sustained by older plants with large canopies and associated leaf areas.

In this study we determined the rate of water table decline that two phreatophytic *Banksia* species (one facultative and one obligate phreatophyte) were able to survive. Two experiments were undertaken; the first was to determine the rate of root elongation for each species during the establishment phase. For this experiment we hypothesised that the species with a distribution restricted to habitats with shallow groundwater would have a slower root elongation rate than the species able survive in deep water table habitats. The second experiment examined the root elongation response of both species to three different rates of water table decline after their roots systems established contact with a consistent water table. We predicted that the response of these older plants to a decline in water table would be to maintain root contact with groundwater at elongation rates equivalent to those observed in the first experiment.

Materials and Methods

Two congeneric species of *Banksia* (Proteaceae) from the Mediterranean-type environment of south-west Australia with contrasting water requirements were studied; *Banksia attenuata* R.Br., a facultative phreatophyte with a wide distribution relative to depth to water table

(Zencich et al. 2002), and *Banksia littoralis* R.Br. an obligate phreatophyte, confined to damplands with shallow depth to groundwater (Arrowsmith 1992; Zencich et al. 2002).

Rhizopod Design

Experiments were undertaken using seedlings grown in rhizopods (*sensu* Mahoney and Rood 1991) in a glasshouse. In experiment 1 we constructed 24 rhizopods using two 2 m tall tubes of 90 mm diameter PVC attached to a reservoir to maintain and manipulate water table depths (n=48 tubes). Tubes were filled with a medium to coarse sand representative of the soil in their natural habitats. The soil had low water holding capacity, with wilting point (-1.5 MPa) and field capacity occurring at 0.04 % and 0.07 % respectively. Each tube contained 1 plant and each species was represented within each rhizopod (i.e. 24 replicates of each species). An additional 5 rhizopods (5 replicate plants per species) were constructed, the tubes of which had a clear polycarbonate wall (covered with black plastic to keep dark) for non-destructive observation of root growth. In experiment 2, 21 rhizopods were used of a similar configuration to experiment 1, giving 21 replicate plants for each of the 2 species.

Experiment 1: Rate of root elongation

To test the first hypothesis seedlings were germinated and transplanted into the rhizopod tubes in March, 8 weeks after sowing when seedlings still had green cotyledons and a maximum of 4 true leaves. The soil was prepared by adding slow release fertiliser (18 g of Osmocote® Native Plants) to the top 5 cm of each tube. Seedlings were watered every 3 days after transplanting for a period of 33 days after which the water table was the only water source available. The temperature of the glasshouse ranged from 18 °C to 25 °C and

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

Seedlings were sampled on the 33rd, 49th, 65th and 92nd day after transplanting with 6 replicates per species per sampling period. Plants were washed free of sand and intact total root and shoot lengths were recorded. Shoots and root portions cut into 20 cm increments were dried at 40 °C until reaching a constant weight. Whole seed weight was determined by weighing 20 individual seeds of each species. Total leaf area was measured prior to drying using a leaf area meter (model Delta-T Type WDIGC-2, Delta T Devices, Cambridge UK.) and leaf water potential ($n = 3$) was determined using a dewpoint potentiometer. Volumetric soil water content and soil water potential (using a dewpoint potentiometer; Decagon Services WP4) were measured at 5 cm increments down the soil profile at each sampling period.

Root elongation rates were determined for destructively sampled seedlings by determining the change in root length from the previous sample period, represented as:

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

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

Root elongation rates for the seedlings in the window tubes were calculated by determining the difference in root length since the previous observation and then dividing by the number of days between observations ($n = 5$). The capillary fringe was determined to be 20 cm above the water table and 80 cm below the soil surface. Window observations of roots were used to determine the number of days it took for each individual to reach 80 cm depth.

155

156 *Plant response to water table decline*

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

170

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

species responses to the treatments. Once the lowering of the water table was completed the seedlings were left in the rhizopods for a further 30 days to allow plants to adjust to the new water table depth. Glasshouse temperatures were controlled and ranged between 18°C and 25°C with an average relative humidity of 50%.

To investigate plant response to the declining water table, transpiration, stomatal conductance and photosynthesis rates were measured within a two hour period during the middle of the day using an infra-red gas analyser (IRGA; Model Li 6400, Li-cor Inc., Lincoln Nebraska). Measurements were made at time 0 (i.e. the day that water table decline commenced) then every 10 days until plants were harvested. For each plant, gas exchange was measured on the youngest fully expanded leaf. Prior to harvesting, predawn plant water potentials of the whole plant were determined using a Scholander-type pressure chamber (Model 3005, PMS instruments, Oregon, USA) as a measure of hydraulic connection to the capillary fringe. Leaf area was determined using an area meter (model Delta-T Type WDIGC-2, Delta T Devices, Cambridge UK) before drying leaves and shoots. Roots were sampled in 20 cm increments, washed free from sand, and then dried and weighed. Volumetric soil water content was determined at 5 cm increments on three occasions for each treatment; the day when the water table decline treatments began, when the treatment ceased and when plants were harvested. Additionally, the water content in millilitres was determined for the top 100 cm of the soil profile.

Data Analysis

To test for differences between study species in the root elongation rate experiment, independent t-tests were performed on each parameter (root elongation rate, root mass, shoot mass, root:shoot ratio, seed weight and pre-dawn plant water potential) at each sampling period. Where data did not meet normality requirements a log transformation was

[Type text]

used. Leaf area and root:shoot ratio data could not be transformed to a normal distribution so non-parametric Kruskal-Wallis tests were used to test for significant differences.

For the response to water table decline experiment, total root and shoot dry weights were compared between each species and treatment using 2-way analysis of variance (ANOVA) and a Tukey *post hoc* test was applied where differences were detected. Leaf area, root:shoot ratios and predawn shoot water potentials could not be transformed to meet the assumptions required for parametric statistical testing, and were therefore analysed using the non-parametric Kruskal-Wallis test. Additionally, the relationship between leaf area and predawn xylem pressure potential was examined using a Spearman's rank order correlation. All analyses were carried out using IBM SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA).

Results

Experiment 1: Rate of root elongation

Soil moisture in the unsaturated zone was initially high, ranging between 1 and 5% throughout the soil profile (Fig. 1). However, after hand watering at the soil surface ceased the soil profile dried progressively from the surface down so that by the conclusion of the experiment, 75% of the unsaturated profile had less than 1% volumetric water content in tubes containing *B. attenuata*. However, in *B. littoralis* tube soil moisture at the end of the experiment was higher with only 40% of the soil profile having less than 1% volumetric water content. Although a significant portion of the unsaturated profile dried during the course of the experiment the water table at 1 m (and the capillary fringe extending 20 cm above it) provided a constant water source for the duration of the experiment.

Seedlings of both study species extended their roots to the capillary fringe by the end of the experiment (92 days after transplanting; Fig. 1) and therefore the total root length attained was not significantly different between species (101.8 ± 3 cm for *B. littoralis* and 95.0 ± 4 cm for *B. attenuata*). Peak elongation rates were observed 8 days after hand watering had stopped, with the facultative phreatophyte *B. attenuata* having a faster rate of root elongation than the obligate phreatophyte, *B. littoralis*. (Fig. 2a and b). This resulted in the *B. attenuata* roots reaching the capillary fringe by day 46 ± 4 whilst the obligate phreatophyte *B. littoralis* took significantly longer at 69 ± 5 days ($t(8) = -4.437$, $p < 0.01$).

The seeds of *Banksia attenuata* had a greater mass (mean weight of 108 ± 4 mg) relative to *B. littoralis* seeds (21 ± 1 mg; Kruskal-Wallis, $\chi^2(40) = 29.268$, $p < 0.001$). By the final sampling period *B. attenuata* had greater root ($t(10) = 4.298$, $p < 0.01$; Table 1) and shoot ($t(10) = 5.423$, $p < 0.001$) mass and twice the leaf area of *B. littoralis* (Kruskal-Wallis, $\chi^2(12) = 5.769$, $p < 0.01$). Cotyledon leaves were larger for *B. attenuata* and remained green for longer (Table 2). By the end of the experiment, the majority of *B. littoralis* seedlings (87%) had brown, shrivelled cotyledon leaves whereas in *B. attenuata* 33% still had green cotyledon leaves. There were no significant interspecific differences in leaf water potential and values were consistent at each sampling period although it was highest at day 33 before the upper parts of the soil profile started to dry.

Experiment 2: Plant response to water table decline

The sandy soil had limited water-holding capacity, with water in the capillary fringe and saturated zone draining rapidly as water table decline occurred (Fig. 3). At the end of the 2nd experiment, the 2 cm day⁻¹ treatment had similar volumetric water content in the soil profile as the 4 cm day⁻¹ and 10 cm day⁻¹ treatments. This is despite the 2 cm day⁻¹ decline taking 40 days to complete, while the 10 cm day⁻¹ treatment took just eight days. Additionally, there

was little change in the soil water content between the end of the water table decline treatment and 30 days after the treatment ended.

There was only limited evidence of a root elongation response following a declining water table in both species, with no roots observed below 120 cm from the soil surface at the end of the experiment. For *B. attenuata*, there did not appear to be any root elongation response to water table decline, with roots remaining in the 80 to 100 cm portion of the soil profile (position of the capillary fringe prior to water table decline) for all rate treatments (Fig. 3). In contrast, the obligate phreatophyte *B. littoralis*, showed a limited response to water table decline in all three treatments with roots extended beyond the pre-decline capillary zone to 120 cm from the surface.

As water content in the top 1 m of the soil profile (the unsaturated vadose zone above the starting water table level) decreased over the course of the experiment, photosynthetic rates and stomatal conductance decreased for both species (Fig. 4). By 30 days after the start of decline all treatments and both species appear to have been disconnected from the water table/capillary fringe with very low rates of photosynthesis observed. Photosynthesis rates dropped rapidly for both species, particularly in the 4 and 10 cm day⁻¹ treatments, taking 10 days to reach less than 6 $\mu\text{mol m}^{-2} \text{s}^{-1}$. However, it took 30 days to reach a similar value in the 2 cm day⁻¹ treatment and corresponds with the longer duration of higher soil water storage in this treatment. Stomatal conductance followed a similar trend with the rate of decline greatest in the 10 cm day⁻¹ water table decline treatment and a significantly slower response for the 2 cm day⁻¹ treatment.

The facultative phreatophyte, *B. attenuata*, had significantly larger leaf area (Kruskal-Wallis $\chi^2(32) = 21.05$, $p < 0.001$) and greater shoot mass (ANOVA $f(32)=34.51$, $p < 0.001$) than the obligate phreatophyte *B. littoralis* across all treatments (Table 3). Additionally, predawn xylem pressure potentials were positively correlated with plant leaf area ($r = 0.772$, $p < 0.001$, $n=31$) with larger leaf area seedlings tending to be more water deficit stressed by the end of the experiment. Higher seedling root and shoot biomass in the 10 cm day⁻¹ treatment (Table 3) is considered a consequence of the greater age of seedlings at the start of treatment (due to the staggered start). Differences and similarities between species however were consistent irrespective of treatment rate.

Discussion

Our hypothesis that the facultative phreatophyte *B. attenuata* has a faster rate of root elongation at the seedling establishment phase than the obligate phreatophyte *B. littoralis*, is supported by the first experiment. The difference in the rate of root elongation between these species may reflect their habitat preferences, e.g. *B. littoralis* confined to habitats that afford year-round access to a comparatively shallow water table (Zencich et al. 2002). In contrast *Banksia attenuata* is distributed across a range of hydrological habitats including xeric environments where there is no access to a water table. In these habitats rapid root elongation deep into the soil profile may be advantageous for seedlings growing on coarse sandy soils in a Mediterranean-type climate, allowing plants to rapidly exploit deeper soil moisture stores during long, dry summers. The greater seed mass of *B. attenuata* also supports this hypothesis and facilitates higher rates of root growth early in the seedling establishment phase.

We predicted that once established, seedlings of the study species would maintain contact with a water table declining at rates similar to the rates of root elongation observed in the

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first experiment; however the study species demonstrated a weak root elongation response to all three rates of water table decline. The notion that the roots of phreatophytic plants are able to extend at such a rate is supported in the literature. Stave et al. (2005) found that *Faidherbia albida* seedlings survived a water table declining at a rate of 5 cm day⁻¹. Similarly, Kranjcec et al. (1998) reported that *Populus balsamifera* maintains contact with a water table declining at 4 cm day⁻¹. The same study also indicated a root growth response following a 10 cm day⁻¹ rate of decline although plant water relations data indicate that this rate was too fast for roots to maintain contact with the water table. Plants in the current study showed similar signs of water stress which was associated with seedlings disconnecting from the water table through lack of root elongation. Having established contact with a consistent water table and increased shoot biomass and leaf area, seedlings in the second experiment were unable to respond to a water table decline at a rate equivalent to the root elongation rate observed prior to establishing contact with the water table.

The initial rate of root elongation by recently germinated seedlings differs to the rate observed for established seedlings (i.e. root contact with water table) responding to a decline in water table. Initial growth by recently germinated seedlings is linked to seed size in many species, with large seeds producing larger seedlings than small seeds (Marshall 1986; Jurado and Westoby 1992; Osunkoya et al. 1993; Leishman and Westoby 1994). In particular, cotyledon reserves of carbon, nitrogen and phosphorus are critical for the early vegetative growth of *Banksia* seedlings in water-limited and nutrient-poor environments (Groom et al. 2001; Lamont and Groom 2013). In this study it was found that *Banksia attenuata* has seeds five times larger and seedlings (3 months old) twice as large as *B. littoralis*. The difference in seed and seedling size between the study species is reflected in the post-emergence root elongation rates with the facultative phreatophyte elongating roots twice as fast as the obligate phreatophyte. However, once seed reserves are exhausted large seededness may no longer influence seedling growth rates, impacting on the ability for

plants to respond to altered environmental conditions such as a decline in the water table (Leishman et al. 2000; Walters and Reich 2000; Dalling and Hubbell 2002). The rapid root elongation rates by young seedlings in the first experiment may be a result of an initial race to develop extensive root systems fuelled by cotyledon reserves. However, the comparative advantage of large seeds did not benefit older seedlings of *B. attenuata* which did not elongate their roots to follow the declining water table.

For the ongoing recruitment and survival of phreatophytes it is important to consider the physiological differences that occur in the lifecycle of the plants. Rapid root elongation by seedlings soon after germination is critical for plants to connect with the water table particularly in water-limited environments. Once their roots are in contact with the water table or capillary fringe above it, phreatophytic plants are likely to adjust their water requirements according to water availability, for example by increasing leaf area (Eagleson 2002). These established plants are able to adapt to some changes in the water table level, for example by adjusting root length in response to the seasonal recharge and decline of an unconfined aquifer (Canham et al. 2012). However, if the magnitude and rate of change is great, plants may become disconnected from the water table if their rate of root elongation is insufficient to maintain contact. High transpirational demand increases the tension of the hydraulic continuum making it susceptible to embolism. The hydraulic gradient is greatest at the root-soil interface and once embolisms occur in the roots, there is a significant decrease in the amount of water able to be transported through the plant resulting in hydraulic failure and plant death (Sparks & Black 1999; McDowell et al. 2008). Thus, the large leaf area afforded by a greater water availability such as a shallow water table, may increase the susceptibility of older plants to rapid decline in the water table.

Like many phreatophytic species globally the *Banksia* study species are being impacted by changes to the hydrological cycle (Naumburg et al. 2005). In the case of south-western Australia declining water tables continue to threaten populations of phreatophytic species and changes in vegetation composition have already been demonstrated (Froend and Sommer 2011). The recruitment of obligate phreatophytic species, such as *B. littoralis* may well depend on the ability of seedlings to develop root systems to access shallow water tables. With progressive increases in depth to groundwater access to this important water source before the onset of the summer dry period may not occur, increasing the risk of water deficits.

In contrast to obligate phreatophytes, facultative phreatophytic species are able to rapidly establish a deep root system to access subsoil moisture and groundwater. *B. attenuata* rapidly develops a deep root system within the first month of seedling growth and therefore can successfully establish in wider range of habitats, including areas with no access to a water table (Zencich et al. 2002). Populations of *B. attenuata* are therefore likely to continue to recruit, relative to *B. littoralis* despite long-term changes in depth to water table. However, our data suggest individuals of *B. attenuata* that have established in habitats with a shallow, consistent water table are vulnerable to rapid drops in the depth of the water table. Although limited to observations made on seedlings, the results of our second experiment suggest mesic antecedent conditions allow these plants to develop large leaf areas and therefore greater transpirational demand. Additionally, there can be intraspecific plasticity in plant hydraulic architecture such as increased vulnerability to xylem embolism associated with populations that have established in wetter habitats (Canham et al. 2009). In combination, these attributes are likely to contribute to increased risk of hydraulic failure in response to a rapidly declining water table, inhibiting the plant's capacity for root elongation and resulting in the disconnection from the water table and plant death.

386

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390 **Conflict of Interest**

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394 interest.

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Tables

Table 1. Summary of plant parameters measured on destructively sampled seedlings for experiment 1. All data are mean \pm 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 \pm 7.4*	600.4 \pm 22.9*
	BL	91.3 \pm 17.2*	231.1 \pm 39.7*
Shoot Weight (mg)	BA	168.0 \pm 19.2	866.7 \pm 72.3*
	BL	156.7 \pm 26.8	360.0 \pm 59.2*
Leaf Area (cm ²)	BA	9.5 \pm 2.2	54.0 \pm 5.7 [†]
	BL	13.7 \pm 1.9	25.6 \pm 5.6 [†]
Pre-dawn Ψ_{leaf} (MPa)	BA	-0.6 \pm 0.1	-1.2 \pm 0.0
	BL	-0.8 \pm 0.1	-1.4 \pm 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

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

Figures

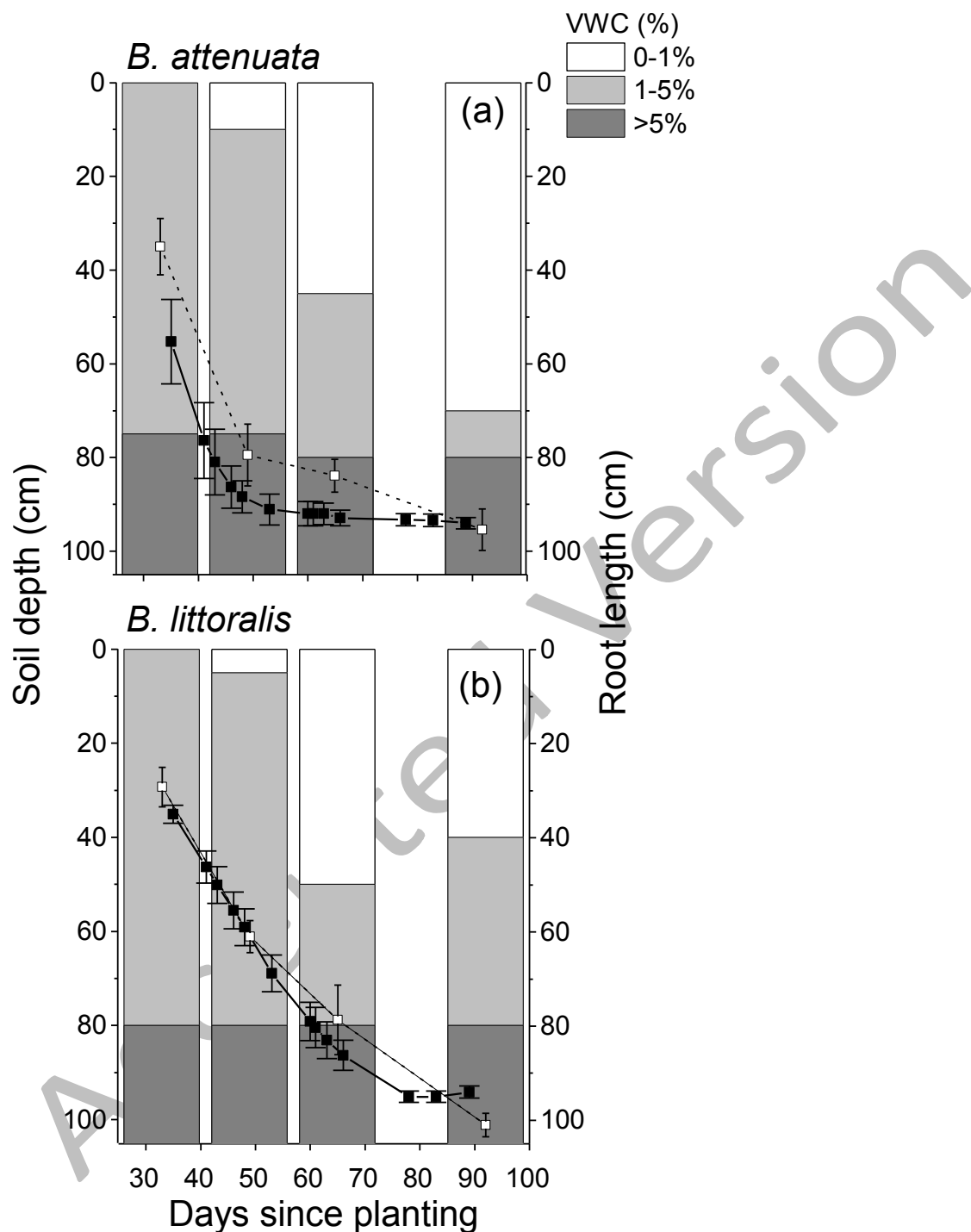


Figure 1. Volumetric water content (VWC; $n = 1$ for both species) and root lengths recorded through observation windows (closed symbols) and destructive sampling (open symbols) for *Banksia attenuata* (top) and *Banksia littoralis* (bottom). Volumetric water content is categorised as 0-1%, 1 to 5% and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

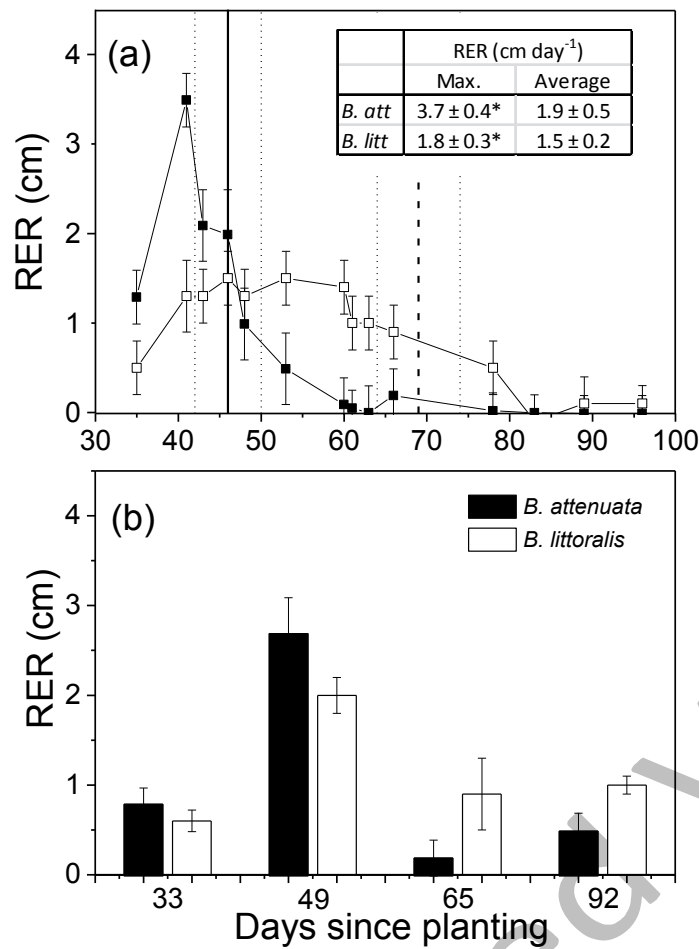


Figure 2. Root elongation rates for *Banksia attenuata* (solid symbols and columns) and *Banksia littoralis* (open symbols and columns) as determined by (a) observation windows and by (b) destructive sampling. Vertical lines in (a) represent the day that roots reached the capillary fringe (80 cm) for *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, $p < 0.05$).

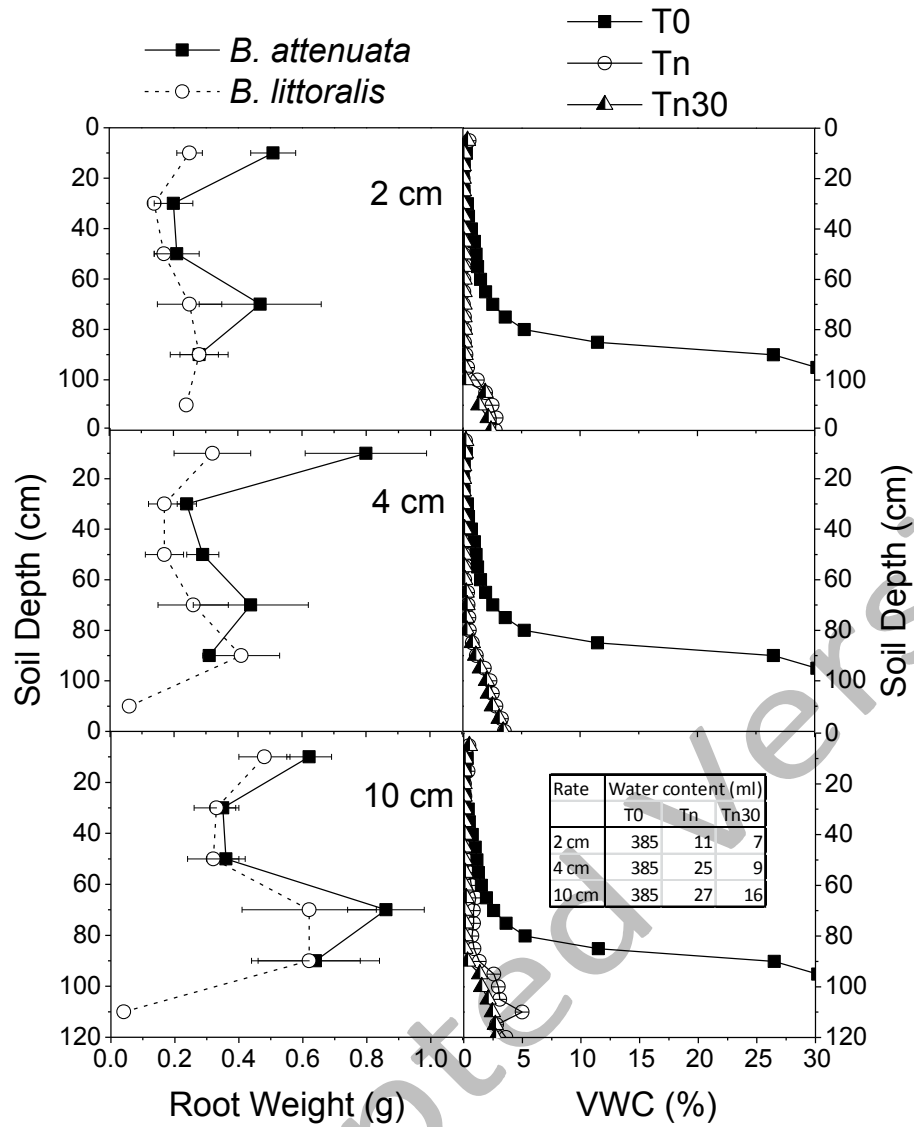


Figure 3. Root dry weights (left) at 20 cm increments for *Banksia attenuata* ($n=4$ for 2 cm day⁻¹ and 4 cm day⁻¹ treatments, and 7 for 10 cm day⁻¹), and *Banksia littoralis* ($n=5$ for 2 cm day⁻¹ treatment and 6 for the 4 cm day⁻¹ and 10 cm day⁻¹) harvested 30 days after 2 cm day⁻¹ 4 cm day⁻¹ and 10 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.

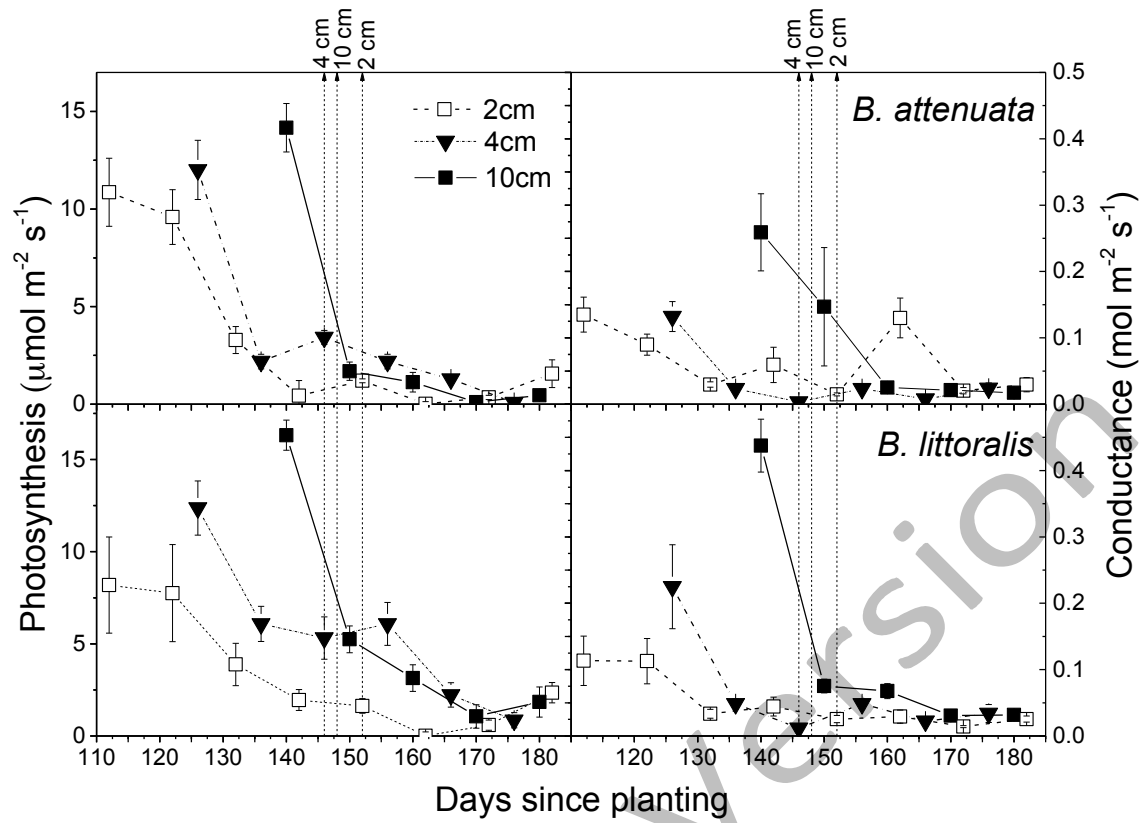


Figure 4. Rate of photosynthesis and stomatal conductance (mean \pm 1 SE) of *B. attenuata* and *B. littoralis* ($n = 7$) seedlings exposed to water table decline of 2 cm, 4 cm and 10 cm per day. The water table drawdown treatment began the day after initial gas exchange measurements. Dashed vertical lines represent the end of the water table drawdown treatment.