

**Tradeoffs between functional strategies for resource-use and drought-  
survival in Mediterranean rangeland species**

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## Abstract

In environments where light is not a limiting resource such as rangelands and grasslands, there is much disagreement regarding the benefits provided by rapid light capture during the growing season and the species' ability to withstand drought during the dry period. In this study, we selected four perennial herbaceous species with contrasting resource-use strategies (acquisitive vs conservative), which were transplanted as monocultures into PVC pots to evaluate their species-specific responses to drought. The two main strategies of drought-survival (avoidance versus tolerance) were driven by distinct underlying mechanisms that allow the plant to delay or tolerate water deficit in leaves. On the one hand, plants that produced reduced leaves with lower surface area:mass ratio (lower SLA) exhibited higher values of leaf water potential (LWP) and leaf relative water content (LRWC), which could be associated to a higher ability to delay tissue dehydration in enlarged leaves. Regarding the below-ground compartment, dehydration avoidance was promoted by prolonged elongation rates of thinner roots that allow the plant to increase water uptake and accessibility during the dry period. On the other hand, dehydration tolerance was positively related with progressive foliage senescence under water deficit, which probably favored a longer survival of meristematic basal tissues. The results presented in this study suggest the existence of a trade-off between the traits favouring rapid light-acquisition and those enhancing the ability to delay leaf dehydration. Thus, the species related most closely with a resource-acquisition strategy (*Bromus erectus* and *Potentilla neumanniana*) could be considered less efficient to delay leaf dehydration than the others (*Carex humilis* and *Festuca christiani-bernardii*), as indicated by their lower values of leaf water potential (LWP) and leaf relative water content (LRWC) under identical conditions of water deficit. Our findings support evidence that there is not a single strategy to effectively cope with drought and reveal the diversity of adaptive mechanisms among coexisting species.

**Keywords** aerial senescence; dehydration avoidance; dehydration tolerance; functional traits;  
root elongation

## **1. Introduction**

In Mediterranean ecosystems, summer drought is considered one of the major factors limiting plant survival, growth and reproduction and, therefore, plant species segregate along natural gradients of soil water availability according to their capacity to withstand drought (Larcher, 2000; Gulías et al., 2002; Valladares and Sánchez-Gómez, 2006; Peñuelas et al., 2011). In the case of Mediterranean perennial herbaceous species, which are subjected to intense water deficit during summer, the ability to remain alive during this period and recover with the first autumn rains is the main adaptative response to ensure plant persistence (Blum, 1996; Volaire et al., 2009).

The ability of a plant species to deal with drought has been associated with specific morphological and physiological traits (Grime, 2001). Two general strategies have been described for delaying or withstanding plant dehydration in perennial plant species (e.g. Levitt, 1980; Ludlow, 1989; Turner, 1997): i) dehydration avoidance, that allows the plant to maintain higher water status for a longer period of time through increased water uptake (Garwood and Sinclair, 1979) or reduced water loss (Voltaire et al., 1998; Martínez-Ferri et al., 2000; Ferrio et al., 2003); and ii) dehydration tolerance, through specific mechanisms that ensure turgor and growth maintenance under moderate drought and reduce damage in basal meristematic tissues under severe drought (West et al., 1990), allowing the plant to recover when rehydration occurs (Bewley, 1995; Voltaire and Lelièvre, 2001). In addition, there are other perennial species such as *Poa bulbosa* L., that exhibit a drought escape strategy through a total summer dormancy involving complete dehydration of the plant during the summer and re-growth when dormancy is released in autumn (Ofir, 1986; Voltaire & Norton, 2006).

Dehydration avoidance and tolerance are clearly not achieved by a single combination of traits (Valladares and Sánchez-Gómez, 2006; Navas et al., 2009), but depend on multiple and different suites of functional traits (Markesteijn, 2010). However, there are very few studies exploring together which traits are best associated with each type of drought-survival strategy, and most of them only consider a limited number of traits (mainly focused on the above-ground compartment). Root traits have been rarely measured in conjunction with leaf traits despite their recognized implication in mechanisms dealing with drought (e.g. Poorter and Markesteijn, 2008; Hernández et al., 2010).

In environments where light is scant, there is a trade-off between species' ability to deal with drought and shade (Smith and Huston, 1989; Niinemets and Valladares, 2006), with those traits favouring plant survival under water scarcity usually constraining light acquisition and thereby plant growth. However, in environments such as rangelands and grasslands, where light is not a limiting resource, there is much disagreement regarding on the benefits provided by rapid light capture during the growing season and the species' ability to withstand drought with the arrival of the dry period (Bazzaz, 1996; Fernández and Reynolds, 2000; Volaire, 2008). Fast-growing species maximise light capture through a resource-acquisition strategy, which is characterised by high values of specific leaf area (SLA) and low-density tissues, whereas opposite attributes are typical of slow-growing species associated with a conservation- resource-use strategy (Chapin et al., 1993; Wright et al., 2004). On the one hand, a larger photosynthetic surface implies higher transpiring leaf area that has been commonly associated to a more wasteful use of water (e.g. Lamont et al., 2002; Escudero et al., 2008). On the other hand, we hypothesized that a higher photosynthetic surface could give the plant a competitive advantage in foraging for water since a greater C acquisition could allow it to maintain higher rates of root elongation during the favourable season and hence to develop deeper and/or more extensive root systems. However, to our knowledge, relationships between the predominant resource-use strategy during the

growing period (acquisitive vs conservative) and the ability to survive summer drought remain largely untested in Mediterranean rangeland species.

In this study, we selected four perennial herbaceous species with contrasting distribution patterns along a natural gradient of soil moisture and depth (Pérez-Ramos et al., 2012) in order to span a wide range of potentially different resource-use strategies. We first measured multiple morphological traits (both above- and below-ground) and quantified root elongation rates to characterize their predominant resource-use strategies (acquisitive vs conservative) under non-limiting water supply. We further evaluated their species-specific responses to prolonged drought under standardized experimental conditions. Dehydration avoidance (i.e., the ability to delay tissue dehydration) was evaluated by monitoring plant water status in response to progressive drought imposition under non-limiting rooting depth conditions. Dehydration tolerance, in contrast, was assessed by recording drought survival in plants transplanted into short pots, discounting for the effect of inter-specific differences in rooting depth on plant water status (*sensu* Volaire and Lelièvre, 2001). This experimental approach enabled us to explore separately the two main types of drought-survival strategies that coexist in environments subjected to intense periods of water deficit. Specifically, we aimed to answer the following questions: i) which morphological leaf and root traits are best associated with dehydration avoidance and tolerance?; ii) is there a trade-off between the traits favouring rapid resource acquisition and those enhancing the ability to deal with drought in Mediterranean rangelands?; and iii) how are these traits and strategies combined in the species studied? By answering these questions, we seek to gain insights into the understanding of different strategies dealing with drought in Mediterranean rangelands and their relationships with distribution patterns within the landscape.

## 2. Material and Methods

### 2.1. Species selection

We selected four perennial herbaceous species, whose distribution strongly segregates along a natural soil depth gradient located in a typical Mediterranean rangeland on the limestone Larzac plateau, 100 km north-west of Montpellier (France). *Festuca christiani-bernardii* K. was the dominant species in shallow (from 20 to 30 cm depth), drier and poorer soils. In contrast, *Bromus erectus* H. and, to a lesser extent, *Carex humilis* L. appeared with higher frequency in deep, moister and more fertile soils. *Potentilla neumanniana* R. showed a more generalist distribution, being similarly abundant along the explored soil depth gradient (Table A.1). To explore phenotypic plasticity, we harvested three different populations of *P. neumanniana* coming from sites with very contrasting conditions of soil moisture (moist, intermediate and dry). However, due to the lack of significant differences between populations, we grouped the three data series for inter-specific comparisons.

### 2.2. Experimental design and plant measurements

In November 2008, a total of 117 plants of each species were collected in the field sourced from at least three different sites, where they were the most abundant species (Table A.1). Tillers or ramets were then randomly separated and transplanted as monocultures into PVC pots filled with a substrate composed of 69.5% sand, 13.2% clay and 17.3% loam, and fertilised before starting the experiment (50 kg/ha N, P and K). We selected this texture for the substrate because it allowed a good drainage and facilitated root harvesting. Three types of pots were used for the different experiments (Exp. from 1 to 4):

- (i) Ten long pots per species (0.75 m height x 0.15 m diameter; 7 plants per pot) were used for measurements of leaf and root traits under non-limiting water supply (Exp. 1) as well as for monitoring plant water status after progressive drought imposition (Exp. 2).

(ii) Three rhizotrons per species, i.e. transparent long tubes (1.10 m height x 0.12 m diameter; 4 plants per pot) covered with opaque film to protect roots from sun radiation and inclined at ~15° from the vertical, were used to measure root elongation rates (Exp. 3).

(iii) Five short pots per species (0.25 m height x 0.15 m diameter; 7 plants per pot) were used to estimate species-specific dehydration tolerances discounting for the effect of inter-specific differences in rooting depth on plant water status (Exp. 4).

Plants were grown in a glasshouse from 4 November 2008 to 10 August 2009 at the CNRS campus in Montpellier (France, 43°38' N, 3°52' E). Mean (minimum – maximum) temperatures within the glasshouse were maintained at 23.5°C (15.7 - 27.2°C) during the day and at 16.2°C (14.8 - 20.7°C) during the night. Saturation vapour pressure deficit (VPD) was 1945±30Pa during the day and 887±8Pa during the night. Global radiation inside glasshouse ranged from 2.2 (January) to 16.4 MJ m<sup>-2</sup> day<sup>-1</sup> (July).

The position of tubes was rotated bi-weekly to avoid the effect of possible small differences in environmental conditions. The pots/tubes were equally watered up 2-3 days per week and maintained at field capacity (~17% soil water content) during 5.5 months. In May 2009, irrigation was stopped in Exp. 2, 3 and 4 in order to analyse responses to drought in each of the species studied. During the drought period, all pots were weighed to determine soil water content (g H<sub>2</sub>O / g dry soil, %) once or twice a week by using the gravimetric method. The soil dry mass was measured in each tube at the end of the experiment after drying at 80°C for 72h. The kinetics of soil water content (SWC) is shown in Figure 1.

#### *2.2.1. Functional traits under non-limiting water conditions (Exp. 1)*

Half of the plants grown in long pots (five monocultures per species) were harvested in May 2009 (just before stopping irrigation) for trait measurements. 12 quantitative traits (five above-ground

and seven below-ground traits) were measured for their known or hypothesized responses to light and soil moisture.

Maximum vegetative height was measured in all individuals (35 per species) using a caliper with precision of 0.1 cm. Water-saturated specific leaf area (SLA; leaf area per unit of dry leaf mass;  $\text{m}^2 \text{kg}^{-1}$ ), leaf dry matter content (LDMC; dry mass per unit of fresh mass;  $\text{mg g}^{-1}$ ) and leaf thickness (LT;  $\mu\text{m}$ ) were determined on 15 fully expanded leaves (three individual leaves per pot and species) following the protocol described by Garnier et al. (2001). Leaf projected area was determined with an area meter (Delta-T Devices, model MK2, Cambridge, UK). Leaf thickness was measured with a linear variable displacement transducer, taking from 5 to 10 measurements per blade depending on the species-specific leaf size. All the leaf samples were weighed, oven-dried at  $60^\circ\text{C}$  for 48h and then re-weighed.

In order to characterize root biomass distribution with depth, all tubes were cut and divided into five sections of variable length (0-10, 10-20, 20-40, 40-60 and 60-75 cm). For each soil section, roots were carefully washed free of soil in water and a representative sub-sample of fresh roots was further scanned at 400 dpi (see Hummel et al., 2007 for methodological details). The image analysis software Winrhizo (ver. 2003b, Regent Instruments Inc., Quebec, Canada) was used to determine length, mean diameter, area and volume of roots (as the sum of the areas and volumes in the different diameter classes). The root material harvested was immediately weighed, oven-dried at  $70^\circ\text{C}$  for 48h and then re-weighed. A number of root functional traits were calculated from these measurements: specific root length (SRL; root length per unit of dry root mass;  $\text{m g}^{-1}$ ), specific root area (SRA; root area per unit of dry root mass;  $\text{cm}^2 \text{g}^{-1}$ ), mean root diameter (mm), tissue mass density (TMD; the ratio of root dry mass to fresh volume;  $\text{g cm}^{-3}$ ) and root dry matter content (RDMC; root dry mass per unit of root fresh mass;  $\text{mg g}^{-1}$ ). For statistical purposes, all these root traits were weighted by the relative biomass of their different soil sections to calculate mean values of the whole root system.



Finally, we calculated the root mass fraction (RMF, root dry mass per unit of total plant dry mass;  $\text{g g}^{-1}$ ), which indicates the proportional biomass investment in the below-ground compartment. Root biomass distribution along the whole soil profile was used to calculate the 95% rooting depth (cm), i. e. the soil depth that contains the 95% of the total dry root biomass.

#### *2.2.2. Species responses to progressive drought (Exp. 2)*

The other half of plants grown in the 0.75 m long pots (five monocultures per species) was used to assess the progressive impact of drought (i.e., dehydration avoidance in leaves). Three complementary descriptors of plant response to drought were measured weekly from the beginning of drought imposition until the end of the experiment (~ 75 days): leaf water potential at predawn (LWP), leaf relative water content (LRWC) and percentage of aerial green biomass (AGB).

LWP was measured on five replicate green leaves per species from separate monocultures with a Scholander-type pressure chamber. LRWC was estimated on 2-5 green leaves from separate monocultures as:  $\text{LRWC} = (\text{FW} - \text{DW}) / (\text{HW} - \text{DW})$ , where FW (fresh weight) was obtained by weighing leaves immediately after harvesting; DW (dry weight) was obtained just after oven-drying the sampling leaves for 48h at 70°C; and HW (weight at full hydration) was measured after full rehydration, i.e. after placing cut end of the leaves in test tubes filled with deionized water during 24h in the dark (Garnier et al., 2001). The percentage of aerial green biomass was assessed visually (scale 0-100%) in the five monocultures of each species.

#### *2.2.3. Quantification of root elongation rates (Exp. 3)*

Root elongation was periodically monitored (weekly during the favourable period and bi-weekly after drought imposition) on rhizotrons (see details above). The trajectory of the root system was recorded on plastic A4-size sheets, directly adhered on the surface of the tube, with permanent

markers; different colors were used for each date. Plastic sheets were scanned and the Winrhizo software (Winrhizo ver. 2003b, Regent Instruments Inc., Quebec, Canada) was used to quantify the accumulated root length on a bi-dimensional plan at each date. The root elongation rate ( $\text{cm cm}^{-2} \text{ day}^{-1}$ ) was measured during the irrigation and the drought periods.

#### 2.2.4. Species survival after rehydration (Exp. 4)

Drought survival was determined on five monocultures per species grown in short pots. When pots achieved similar conditions of severe drought (i.e., SWC values ranging from 2 to 3%), monocultures were separately rehydrated and maintained under full irrigation during 10-12 days. Drought survival was estimated as the percentage of plants recovering (aerial leaf growth observed visually) in each monoculture after full rehydration. Drought survival under these constrained and similar rooting depth conditions of all species was used to analyse standardised dehydration tolerance of surviving organs. Since plants could not exhibit a dehydration avoidance strategy through an increased water uptake with deeper root systems, species-specific strategies of drought tolerance could be comparable (Voltaire and Lelièvre, 2001).

#### 2.3. Data analyses

Inter-specific differences in the 14 functional traits measured under non-limiting water conditions were evaluated using an analysis of variance (*one-way* ANOVA) and the post-hoc Tukey test.

The response of the four studied species to progressive drought was assessed using two complementary approaches. Since the rate of decrease in soil water content varied between species due to differential rates of soil water uptake (Figure 1), inter-specific differences in the three response-variables (LWP, LRWC and AGB) were evaluated along the continuous gradient of soil water. In the first approach, these three response-variables were modelled independently as a function of SWC, using maximum likelihood techniques (Edwards, 1992). We tested three

alternative functions (linear, exponential and Michaelis-Menten), that cover a wide range of possible forms (see equations in Table A.2). Competing models were selected with the Akaike Information Criterion corrected for small sample sizes ( $AIC_c$ ) (Burnham and Anderson, 2002) as a measure of goodness of fit: the lower the AIC value, the better the model. The  $R^2$  of the regression of observed vs. predicted was used as a quantitative measure of goodness of fit of each alternative model. To determine whether the four studied species responded differently to drought, we compared the 95% confidence intervals of the slopes ( $b$  parameter) and intercepts ( $a$  parameter) of the species-specific models. When 95% support intervals did not overlap, differences between species were considered to be relevant.

In the second approach, three categories of SWC values were considered:  $SWC \leq 3\%$ ,  $3\% < SWC < 5\%$  and  $SWC \geq 5\%$ . Inter-specific differences in the three response-variables for each of these three categories were tested by means of an analysis of variance (*one-way* ANOVA) and the post-hoc Tukey test.

Differences between species in root elongation rates were assessed using the *one-way* ANOVA and the post-hoc Tukey test, the data series being previously separated at four different intervals of soil depth: 0-30, 30-60, 60-90 and 90-110 cm. These statistical analyses were carried out separately for the two contrasting periods of the experiment: under full irrigation (from November 2008 to May 2009) and after drought imposition (from May to July 2009).

Inter-specific differences in plant survival after full irrigation (dehydration tolerance) were evaluated using the *one-way* ANOVA and the post-hoc Fisher test. Finally, Pearson's correlation analyses were conducted to identify which morphological leaf and root traits were best associated with dehydration avoidance (LWP, LRWC and AGB,) and tolerance (plant survival after rehydration).

The models were implemented using the *likelihood* package version 1.1 for R and software written specifically for this study in R v 2.5.0 (R Development Core Team 2006). The rest of statistical analyses were carried out using STATISTICA version 6.0 (StatSoft Inc., 2001). Before these analyses, those variables not normally distributed were log-, square-root- or arcsine-transformed to fulfil assumptions of normality and homoscedasticity. Normality was tested using the Kolmogorov–Smirnov test.

### 3. Results

#### 3.1. Functional traits under non-limiting water conditions

The four studied species showed strong differences in most of the morphological traits quantified in this study. With regard to the above-ground traits, *B. erectus* and *P. neumanniana* could be related most closely with a resource-acquisition strategy as they had larger leaves of higher SLA and lower thickness in comparison with the other two species (Table 1 and Fig. 2). In contrast, *C. humilis* and *F. christiani-bernardii* produced smaller and thicker leaves with low transpiring surface per unit of dry mass, three leaf attributes commonly associated to a resource-conservation strategy (Table 1 and Fig. 2). The four species also showed significant differences in plant height, *B. erectus* being the tallest and *P. neumanniana* the shortest (Table 1).

Regarding the below-ground compartment, *B. erectus* exhibited the deepest root system (95% rooting depth); its roots had relatively low values of tissue density and root dry matter content (Table 1 and Fig. 2). Opposite attributes characterized *C. humilis*, which showed the shallowest and most sclerophyllous (i.e., the highest values of RDMC) root system (Table 1 and Fig. 2). *F. christiani-bernardii* exhibited the largest root foraging ability in superficial soil layers as indicated by their higher values of SRL and SRA, probably resulting from their finer and less dense roots. Finally, *P. neumanniana* had intermediate values for all the measured root traits (Table 1).

Under non-limiting water supply, *B. erectus* exhibited 2-3 times higher rates of root elongation (RER) than the other three species (Table 1 and Figure 3a), but these differences were more pronounced in deeper soil layers (Figure 3b). *P. neumanniana* developed a root system homogeneously distributed along the entire depth gradient, equalling to *B. erectus* in the two deepest soil layers (Figure 3b). The other two species showed high root elongation rates in the most superficial layer, that were diminishing with increasing depth (Figure 3b). In fact, the roots of these two species remained shallower than those of *B. erectus* and *P. neumanniana* during the period of full irrigation (Table 1).

### 3.2. Species responses to progressive drought

The studied species differed substantially in their responses to progressive drought (i.e., decreasing SWC) (Figure 4 and Table A.2).

The percentage of aerial green biomass (AGB) decreased with increasing drought in the four studied species following a Michaelis-Menten function (Table A.2). *B. erectus* showed the greatest proportion of senescent tissues under water deficit (with an AGB reduction up to 77%), followed by *P. neumanniana* (up to 61%) and *F. christiani-bernardii* (up to 55%; Figure 4a). In contrast, *C. humilis* retained the highest proportion of green leaves over a longer time (with an AGB reduction up to 33%; Figure 4a).

Leaf water potential (LWP) diminished exponentially with increasing drought in the four species (Table A.2). However, the decrease in LWP with increasing drought varied strongly between species as indicated by the lack of overlapping between confidence intervals of both equation parameters (Table A.2). Interestingly, these inter-specific differences were only significant for intermediate values of the drought (SWC ranging from 3 to 5%), with *F. christiani-bernardii* exhibiting the least negative values of LWP (Figure 4b).

Leaf relative water content decreased linearly with increasing drought, but the magnitude of decrease differed substantially between species (Table A.2). The magnitude of the decrease in LRWC was greater in *B. erectus* (as indicated by a steeper slope, i.e. larger *b* parameter), followed by *P. neumanniana*, *F. christiani-bernardii* and finally *C. humilis* (Table A.2). The species ranking remained constant at the low ( $SWC \leq 3\%$ ) and intermediate values of SWC (from 3 to 5%), *B. erectus* and *P. neumanniana* showing again the lowest values of LRWC compared with *F. christiani-bernardii* and *C. humilis* (Figure 4c).

After drought imposition, root elongation rates of *B. erectus*, *P. neumanniana* and *C. humilis* decreased strongly, whereas *F. christiani-bernardii* lengthened its root system over a longer time (Figure 3a). Particularly, this species exhibited higher values of RER during the drought period compared with other species (except for *B. erectus*), although these differences were only significant at intermediate soil layers (30-60 and 60-90 cm; Figure 3c).

In summary, the two species related most closely with a resource-acquisition strategy (*B. erectus* and, to a lesser extent, *P. neumanniana*) showed the highest levels of water stress in leaves, as indicated by their lower values of LWP and LRWC under comparable conditions of water deficit (i.e., for SWC values ranging from 3 to 5%; Figure 4). In addition, these two species tended to senesce earlier, as a possible adaptation to ensure a longer survival of meristematic basal tissues. Some morphological traits measured under non-limiting water conditions were correlated with the plant's ability to maintain hydration in leaves during drought (Table 2 and Fig. 2). Specifically, LWP and LRWC were both negatively correlated with leaf size and specific leaf area (Figures 5a, 5b, 5c). Regarding the below-ground component, LWP was negatively related with root diameter (Figure 5e) and positively with RER quantified during the drought period (Figure 5d). Interestingly, the species with deeper root systems and higher root elongation rates during the irrigation period exhibited the lower aerial green biomass (Table 2), i.e. the highest rates of senescence of leaves after drought imposition.

### 3.3. Species survival after rehydration

All species were able to recover with rehydration after a period of severe drought (Figure 6). The percentage of plants recovering after full rehydration differed substantially between species, with the two Poaceae (*B. erectus* and *F. christiani-bernardii*) having the highest plant survival (57.1 and 28.6%, respectively; Figure 6).

Interestingly, this measurement of dehydration tolerance was positively related with active foliage senescence, as indicated by its negative correlation with AGB under moderate drought (Table 2 and Figures 2 and 5f). However, plant survival after rehydration was not significantly associated with LWP and LRWC (Table 2). In addition, dehydration tolerance was positively correlated with rooting depth measured in long pots (Table 2).

## 4. Discussion

### 4.1. Functional traits associated with dehydration avoidance and tolerance

The results from this study suggest that several morphological traits measured under non-limiting water conditions could be useful to predict plant responses under subsequent drought imposition (see Fig. 2). With regard to the above-ground traits, plants that produced reduced leaves with lower surface area:mass ratio (lower SLA) exhibited higher values of leaf water potential (LWP) and leaf relative water content (LRWC), which could be associated to a higher ability to delay tissue dehydration in enlarged leaves. These types of leaves, usually comprising small and thickened cells (Garnier & Laurent, 1994; Poorter et al., 2009), probably allow the plant to reduce water use and avoid the loss of turgor at low soil water potentials (Zimmermann, 1978; Witkowski & Lamont, 1991; Dudley, 1996). These results support previous studies reporting that SLA strongly decreases with increasing drought (e.g., Salleo & Lo Gullo, 1990, Carter et al., 1997, Yin, 2002; Poorter et al., 2009). Regarding the below-ground compartment, plants with thinner roots

were more dehydration-avoiders, likely because a lower root diameter usually involves a higher hydraulic conductivity and consequently a higher ability of water uptake (Rieger & Litvin, 1999). In addition, the capacity to lengthen root growth during the drought period was identified as one of the major mechanisms to delay leaf dehydration. In arid or semiarid natural environments, soil water content commonly increases with soil depth during the dry season (Engelbrecht et al., 2005). Thus, a relatively small proportion of roots in deeper soil layers can be crucial in extracting additional water and thereby maintaining higher levels of plant water over a longer period of time (Jackson et al., 1996; Nicotra et al., 2002).

Dehydration tolerance, measured as drought survival under limited rooting depth conditions, was positively related with progressive foliage senescence under water deficit. Thus, the most dehydration-tolerant species progressively shed most of their leaves as drought intensified, probably as a mechanism for reducing the transpiring leaf surface and thereby the rate of water loss in meristems (Volaire et al., 1998a; Volaire and Lelièvre, 2001; Munne-Bosch & Alegre, 2004). The maintenance of turgor and membrane stability in basal meristematic tissues under severe drought has been identified as a key adaptive mechanism to ensure plant persistence until the arrival of the first rains in Mediterranean perennial plant species (Blum, 1996; Volaire et al., 2009). Previous studies have shown that the plant's ability to survive under high soil dehydration is commonly associated with protection and repair mechanisms that preserve the structural integrity of cell membranes in meristematic tissues (Bewley, 1995). However, these underlying mechanisms are not necessarily related with the morphological traits quantified in this study. Further studies are therefore necessary to better understand which specific mechanisms and traits allow the tolerant species to prevent or minimize damage in tissues caused by severe drought and recover when rehydration occurs.

Our results suggest that the two main strategies to deal with drought in perennial plant species (avoidance *versus* tolerance) need to be analysed cautiously when plant survival is considered.



Thus, the ability to recover with rehydration was less pronounced in those species more dependent upon dehydration-avoidance. This resulted in a trade-off in drought survival between dehydration tolerance of meristems and dehydration avoidance in leaves. Our study suggests the implication of extensive leaf senescence as a water-saving strategy for meristematic basal tissues and highlights the importance of exploring separately dehydration tolerance and avoidance strategies due to their different functional associations with morphological traits (Fig. 2).

#### 4.2. Is there a trade-off between resource-use and drought-survival strategies?

The results presented in this study suggest the existence of a trade-off between the traits favouring rapid light-acquisition and those enhancing the ability to delay leaf dehydration (Fig. 2).

The species related most closely with a resource-acquisition strategy (*B. erectus* and *P. neumanniana*) exhibited higher levels of water stress in leaf than the other two species, as indicated by their lower values of LWP and LRWC under identical conditions of water deficit. These two species progressively lost most of their leaves as drought increased, likely as a mechanism for reducing water loss and ensuring a longer survival of meristematic basal tissues (Volaire et al., 1998a; Volaire and Lelièvre, 2001; Munne-Bosch & Alegre, 2004). According to our initial hypothesis, the higher potential to capture light provided by a larger photosynthetic leaf area conferred on the plants a competitive advantage in foraging for water in deep soil layers through the maintenance of higher root elongation rates during the favourable season. In spite of their deeper root systems, these fast-growing species exhibited greater levels of water stress in leaf below a threshold value of soil water and, in this sense, they could be considered less efficient at delaying leaf dehydration than the species exhibiting a resource-conservation strategy. In Mediterranean ecosystems, a deeper root system does not always imply a plant's greater ability to cope with drought (Joffre et al., 2001 and references therein). Thus, shallow roots could be more efficient in water-limited and less-productive sites (Schenk and Jackson, 2002) since the

greater energy costs for construction, maintenance and resource uptake that implies a deep root system may be non-viable or wasteful in this type of habitat (Adiku et al., 2000).

In contrast, the species exhibiting a resource-conservation strategy (*C. humilis* and *F. christiani-bernardii*) showed a water-saving strategy in leaves, probably because leaves with lower SLA allow the plant to reduce the transpiring leaf area (i.e., water loss) and maintain turgor, photosynthetic activity and carbon gain over a longer period of time (Givnish, 1987; Lamont et al., 2002; Escudero et al., 2008). Interestingly, these species mostly captured water from upper soil layers during the favourable season, but they lengthened their root systems over a longer time during the drought period. Vertical root distributions can change greatly in response to shifts in vertical distributions of soil water, such as drying of the soil surface (Klepper et al., 1991; Wraith and Wright, 1998). Our findings support previous studies reporting large between-species differences in the minimum value of soil water at which roots elongate, with the lowest values appearing in those species better-adapted to drought (Schenk, 2005).

Conversely to the results found with regard to leaf dehydration avoidance, we did not find evidence for a trade-off between resource-use and dehydration-tolerance strategies. Thus, species exhibiting an acquisitive (as *B. erectus*) and a conservative strategy (as *F. christiani-bernardii*) were both highly dehydration-tolerant, as indicated by their high values of plant survival after rehydration. Our results suggest that the underlying mechanisms of dehydration tolerance could be also associated with the protection of aerial meristems by sheaths, as commonly found in graminoid species (Wilman et al., 1994)

#### 4.3. Drought-survival strategies and local distribution patterns along a moisture gradient

While it is essential to define strategies, it is recognized that interpreting the behaviour of native plant species in terms of a single response could be quite misleading since plant communities may exhibit in nature a wide range of combined responses to withstand drought (Ludlow, 1989).

Our findings support evidence that there is not a single strategy to effectively survive drought and reveal the diversity of adaptive mechanisms among coexisting species.

We identified three different but non-exclusive drought-survival strategies in the study area (see Fig. 2): (1) the species exhibiting a resource-acquisition strategy (*B. erectus* and *P. neumanniana*) delayed meristem dehydration through increased root foraging ability in deeper soil layers and senescence of most aerial biomass with increasing drought; (2) the species exhibiting a resource-conservation strategy (*C. humilis* and *F. christiani-bernardii*) tried to delay leaf dehydration through reduced and more sclerophyllous leaves and thinner roots with prolonged elongation rates during the dry period; and (3) the two Poaceae species (*B. erectus* and *F. christiani-bernardii*) exhibited high dehydration tolerance abilities. These results are in accordance with previous studies suggesting that most plants of water-limited ecosystems have developed mechanisms of drought-avoidance, whereas the ability to tolerate water deficit is only restricted to certain functional groups (Levitt, 1980; Valladares et al., 2004).

The identification of drivers and strategies to deal with drought is of great interest since they could help to explain patterns of local and regional species distribution and predict the vulnerability of communities to future environmental scenarios (McDowell et al., 2008; McDowell, 2011). In our case, the predominant drought-survival strategy could be related, at least partially, to species habitat and local distribution patterns in the study area. The species identified with the first drought-survival strategy (particularly *B. erectus*, the least efficient species at delaying leaf dehydration) seem to be more efficient in moister and deeper soils, which maintain greater values of soil humidity over a longer time during the dry season. Conversely, one of the species categorised within the second drought-survival strategy (*F. christiani-bernardii*), which was highly dehydration-tolerant and exhibited the greatest ability to delay leaf dehydration, was the most abundant one in the dry extreme of the soil moisture gradient. However, the relatively high frequency of *C. humilis* in moister and deeper soils or the more generalist distribution of *P.*

*neumanniana* suggest than, in addition to drought-survival mechanisms, other factors such as competitive ability, resistance against herbivory or dispersal limitation could also shape the distribution patterns of Mediterranean perennial species along the soil moisture gradient (Poorter and Markesteijn, 2008).

The results of our experimental approach suggest the existence of a strong diversification of strategies to deal with drought over very short distances, which were driven by distinct underlying mechanisms that allow the plant to delay or tolerate water deficit in leaf or aerial meristems. The large diversity of adaptive strategies to survive drought could be interpreted as a potential mechanism for favouring species coexistence and promoting the maintenance of highly diverse rangeland communities.

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**Table 1.** Mean ( $\pm$ SE) values of the above- and below-ground traits (Exp. 1) as well as the root elongation rates (Exp. 3) measured in this study under non-limiting water conditions. Different letters indicate significant differences between species in accordance with the post-hoc Tukey test ( $P < 0.05$ ). SLA: specific leaf area; LDMC: leaf dry matter content; RMF: root mass fraction; RDMC: root dry matter content; SRL: specific root length; SRA: specific root area; TMD<sub>r</sub>: Tissue mass density of the root.

| Functional traits   | <i>Bromus erectus</i> | <i>Carex humilis</i>          | <i>Festuca christiani-bernardii</i> | <i>Potentilla neumanniana</i> |
|---|-----------------------|-------------------------------|-------------------------------------|-------------------------------|
| <u>Above-ground traits</u>                                  |                       |                               |                                     |                               |
| Plant height (cm)   | 18.70 $\pm$ 1.40 a    | 6.67 $\pm$ 0.17 b             | 8.36 $\pm$ 0.48 b                   | 2.05 $\pm$ 0.08 c             |
| Leaf size (cm <sup>2</sup> )                                | 2.44 $\pm$ 0.26 a     | 0.52 $\pm$ 0.04 b             | 0.24 $\pm$ 0.02 b                   | 1.93 $\pm$ 0.08 c             |
| SLA (cm <sup>2</sup> g <sup>-1</sup> )                      | 165.15 $\pm$ 5.64 a   | 111.01 $\pm$ 2.49 b           | 62.10 $\pm$ 1.31 c                  | 149.59 $\pm$ d                |
| LDMC (mg g <sup>-1</sup> )                                  | 337.19 $\pm$ 14.76 a  | 327.06 $\pm$ 4.25 a           | 314.91 $\pm$ 4.04 a                 | 269.26 $\pm$ 4.08 b           |
| Leaf thickness ( $\mu$ m)                                   | 550.45 $\pm$ 13.96 a  | 762.65 $\pm$ 65 $\pm$ 21.91 b | 590.77 $\pm$ 44.48 a                | 544.84 $\pm$ 4.69 a           |
| <u>Below-ground traits (Exp. 1)</u>                         |                       |                               |                                     |                               |
| RMF (g g <sup>-1</sup> )                                    | 0.45 $\pm$ 0.06 a     | 0.45 $\pm$ 0.03 a             | 0.45 $\pm$ 0.01 a                   | 0.38 $\pm$ 0.02 a             |
| 95% rooting depth (cm)                                      | 53.98 $\pm$ 6.10 a    | 26.70 $\pm$ 2.28 b            | 40.46 $\pm$ 2.83 b                  | 38.67 $\pm$ 2.44 b            |
| RDMC (mg g <sup>-1</sup> )                                  | 143.11 $\pm$ 6.87 a   | 252.20 $\pm$ 16.08 b          | 158.30 $\pm$ 11.66 a                | 209.44 $\pm$ 11.25 a          |
| SRL (m g <sup>-1</sup> )                                    | 123.01 $\pm$ 13.02 a  | 163.62 $\pm$ 19.53 a          | 381.21 $\pm$ 26.84 b                | 116.04 $\pm$ 8.12 a           |
| SRA (cm <sup>2</sup> g <sup>-1</sup> )                      | 361.59 $\pm$ 27.46 a  | 426.93 $\pm$ 51.25 a          | 881.41 $\pm$ 61.74 b                | 307.33 $\pm$ 18.83 a          |
| Root diameter (mm)  | 0.30 $\pm$ 0.01 a     | 0.28 $\pm$ 65 $\pm$ 0.005 ab  | 0.23 $\pm$ 0.0008 b                 | 0.27 $\pm$ 0.003 ab           |
| TMD <sub>r</sub> (g cm <sup>-3</sup> )                      | 0.13 $\pm$ 0.004 ab   | 0.14 $\pm$ 0.018 ab           | 0.07 $\pm$ 0.005 b                  | 0.21 $\pm$ 0.01 a             |
| <u>Below-ground traits (Exp. 3)</u>                         |                       |                               |                                     |                               |
| Maximum root depth (cm)                                     | 118.8 $\pm$ 0.00 a    | 69.83 $\pm$ 4.40 b            | 76.07 $\pm$ 9.50 b                  | 118.64 $\pm$ 0.11 a           |
| Root elongation rate (cm cm <sup>-2</sup> d <sup>-1</sup> ) | 0.015 $\pm$ 0.003 a   | 0.005 $\pm$ 0.002 b           | 0.006 $\pm$ 0.002 bc                | 0.007 $\pm$ 0.001 c           |

**Table 2.** Matrix of correlations among the 12 functional traits measured under non-limiting water supply, the root elongation rates, the percentages of plant survival after rehydration and the three response-variables used for monitoring plant responses to water deficit at moderate drought (i.e. for SWC values ranging from 3 to 5%). The significance level is indicated as follows: \*\*\*P<0.001; \*\*P<0.01; \*P<0.05. Significant values of “r” have been highlighted with bold letters.

|  | Aerial green biomass | Leaf water potential at predawn | Lamina relative water content | Plant survival after rehydration |
|--|----------------------|---------------------------------|-------------------------------|----------------------------------|
| Plant height                             | -0.79                | -0.44                           | -0.27                         | 0.92                             |
| Leaf size                                | -0.83                | <b>-0.95*</b>                   | <b>-0.99**</b>                | 0.67                             |
| Specific leaf area                       | -0.66                | <b>-0.99**</b>                  | <b>-0.94*</b>                 | 0.49                             |
| Leaf dry matter content                  | -0.27                | -0.03                           | 0.28                          | 0.51                             |
| Leaf thickness                           | 0.69                 | 0.36                            | 0.65                          | -0.55                            |
| Root mass fraction                       | -0.12                | 0.24                            | 0.49                          | 0.39                             |
| 95% rooting depth                        | <b>-0.95*</b>        | -0.54                           | -0.63                         | <b>0.93*</b>                     |
| Root dry matter content                  | 0.76                 | 0.14                            | 0.27                          | -0.81                            |
| Specific root length                     | 0.36                 | 0.87                            | 0.78                          | -0.19                            |
| Specific root area                       | 0.32                 | 0.86                            | 0.77                          | -0.14                            |
| Root diameter                            | -0.65                | <b>-0.93*</b>                   | -0.73                         | 0.59                             |
| Tissue mass density (root)               | -0.02                | -0.59                           | -0.67                         | -0.23                            |
| Root elongation rate (irrigation period) | <b>-0.98*</b>        | -0.77                           | -0.69                         | <b>0.98</b>                      |
| Root elongation rate (drought period)    | 0.26                 | <b>0.88*</b>                    | 0.68                          | -0.10                            |
| Aerial green biomass                     | -                    | 0.75                            | 0.76                          | <b>-0.96*</b>                    |
| Leaf water potential at predawn          | -                    | -                               | <b>0.92*</b>                  | -0.61                            |
| Lamina relative water content            | -                    | -                               | -                             | -0.56                            |

## Figure legends

**Figure 1.** Temporal dynamics of soil water content for the four studied species after drought imposition (Exp. 2).

**Figure 2.** Summary diagram on relationships among drought-survival strategies (dehydration avoidance and tolerance) and resource-use strategies (resource-acquisition versus resource-conservation) mediated by morphological traits and root elongation rates. Connecting solid lines indicate which species significantly showed the highest values of each of the main morphological traits quantified in this study. Connecting arrows indicate significant relationships (positive with solid lines and negative with dotted lines) among morphological traits, root elongation rates and the four response-variables used for monitoring plant responses to water deficit (i.e., leaf water potential, leaf relative water content, aerial green biomass and plant survival after rehydration). Codes: SLA: specific leaf area; LDMC: leaf dry matter content; RDMC: root dry matter content; SRL: specific root length; SRA: specific root area;  $TMD_r$ : tissue mass density of the root;  $RER_{irr}$ : root elongation rate during the irrigation period;  $RER_{drought}$ : root elongation rate during the drought period.

**Figure 3.** Root elongation dynamics of the four studied species: temporary dynamics for the whole root system (panel A); and separating in four selected ranges of soil depth (0-30, 30-60, 60-90 and 90-110 cm), both under the period of full irrigation (panel B) and after drought imposition (panel C). Vertical bars indicate standard-error values.

**Figure 4.** Species-specific responses to drought of the four studied species for three selected ranges of soil water content (SWC; below 3%, from 3 to 5%, and above 5%). Plant water status was evaluated by periodically monitoring: percentage of aerial green biomass (panel A), leaf water potential at predawn

(panel B) and leaf relative water content (panel C). Different letters indicate significant differences among species in accordance with the post-hoc Tukey test ( $P < 0.05$ ). Species codes are: Bro: *Bromus erectus*; Car: *Carex humilis*; Fes: *Festuca christiani-bernardii*; Hel: *Helianthemum apenninum*; and Pot: *Potentilla nemanianna*.

**Figure 5.** Leaf and root traits best associated with plant water stress (under identical conditions of soil water deficit,  $3 < \text{SWC} < 5\%$ ) for the four species included in this study: leaf size (panels A and C), specific leaf area (panel B), root elongation rate after drought imposition (panel D), root diameter (panel E). Relationship between aerial green biomass under moderate drought ( $3 < \text{SWC} < 5\%$ ) and mean survival after full rehydration (panel F). The significance level is indicated as: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Species codes as in Figure 2.

**Figure 6.** Mean percentage of plants recovering after 10-12 days of full rehydration for the four studied species. Different letters indicate significant differences between species in accordance with the post-hoc Fisher test ( $P < 0.05$ ). Species codes as in Figure 2.

Fig. 1

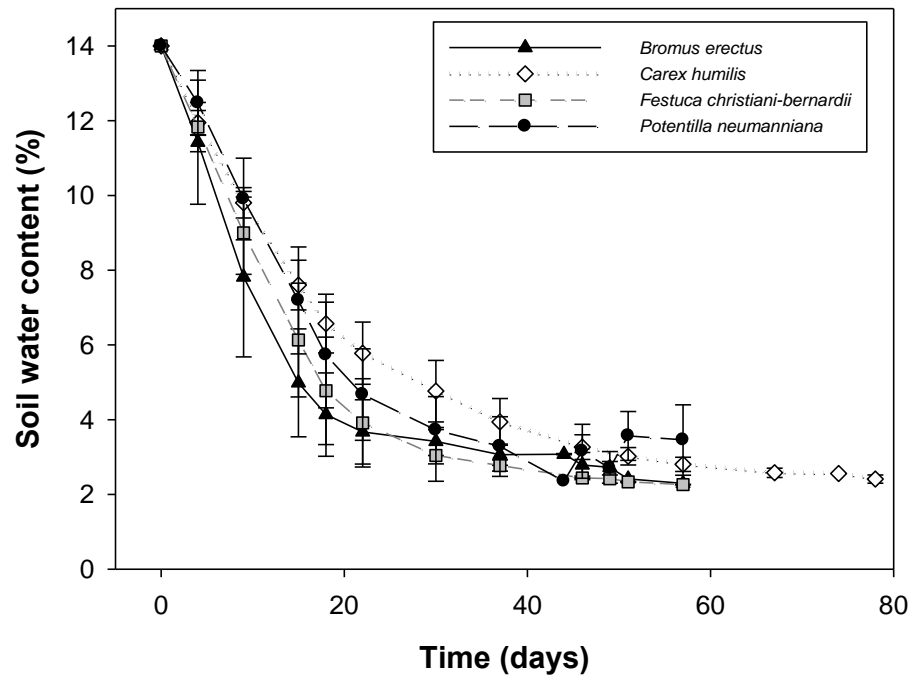


Fig. 2.

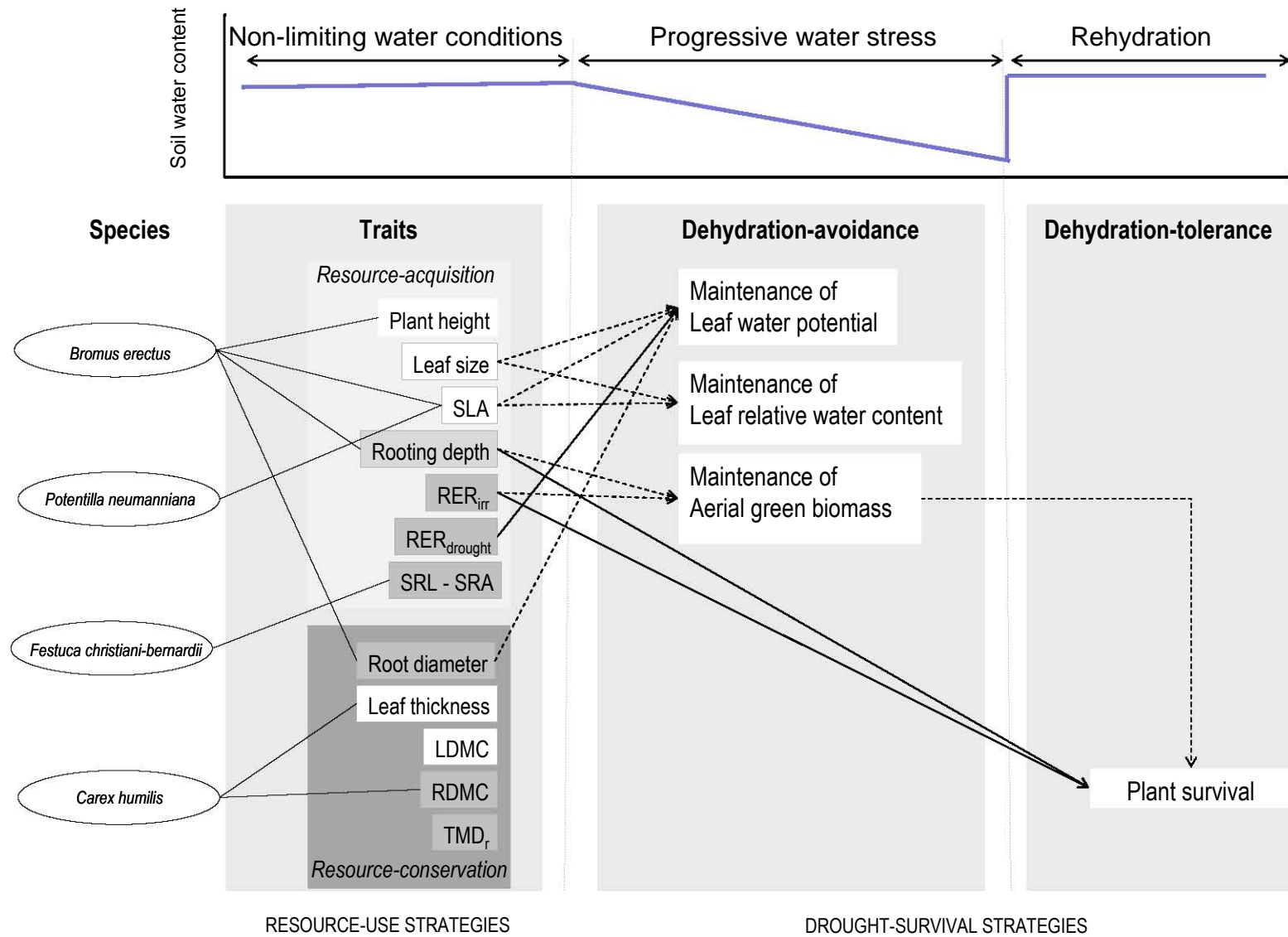




Fig. 3

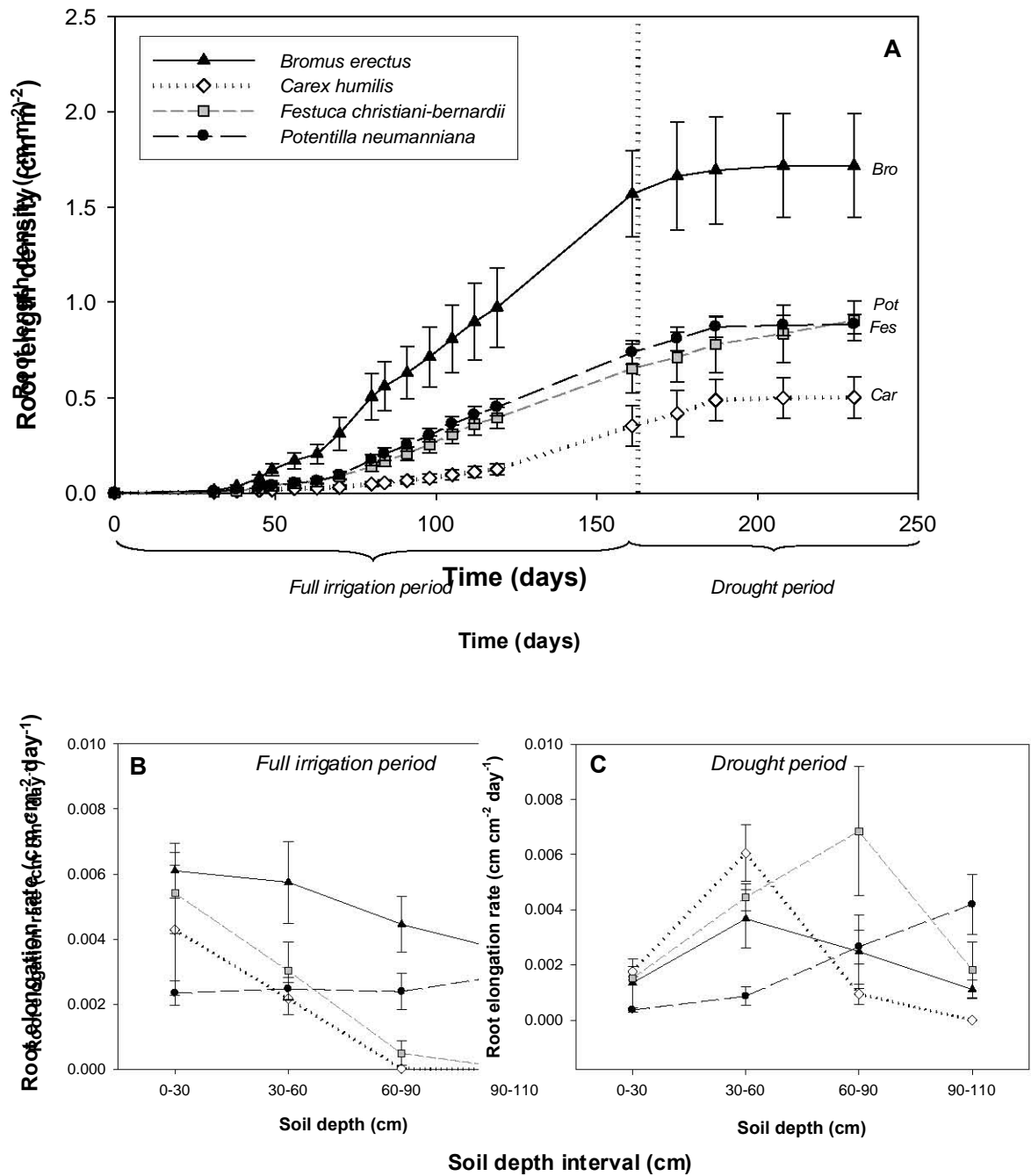


Fig. 4

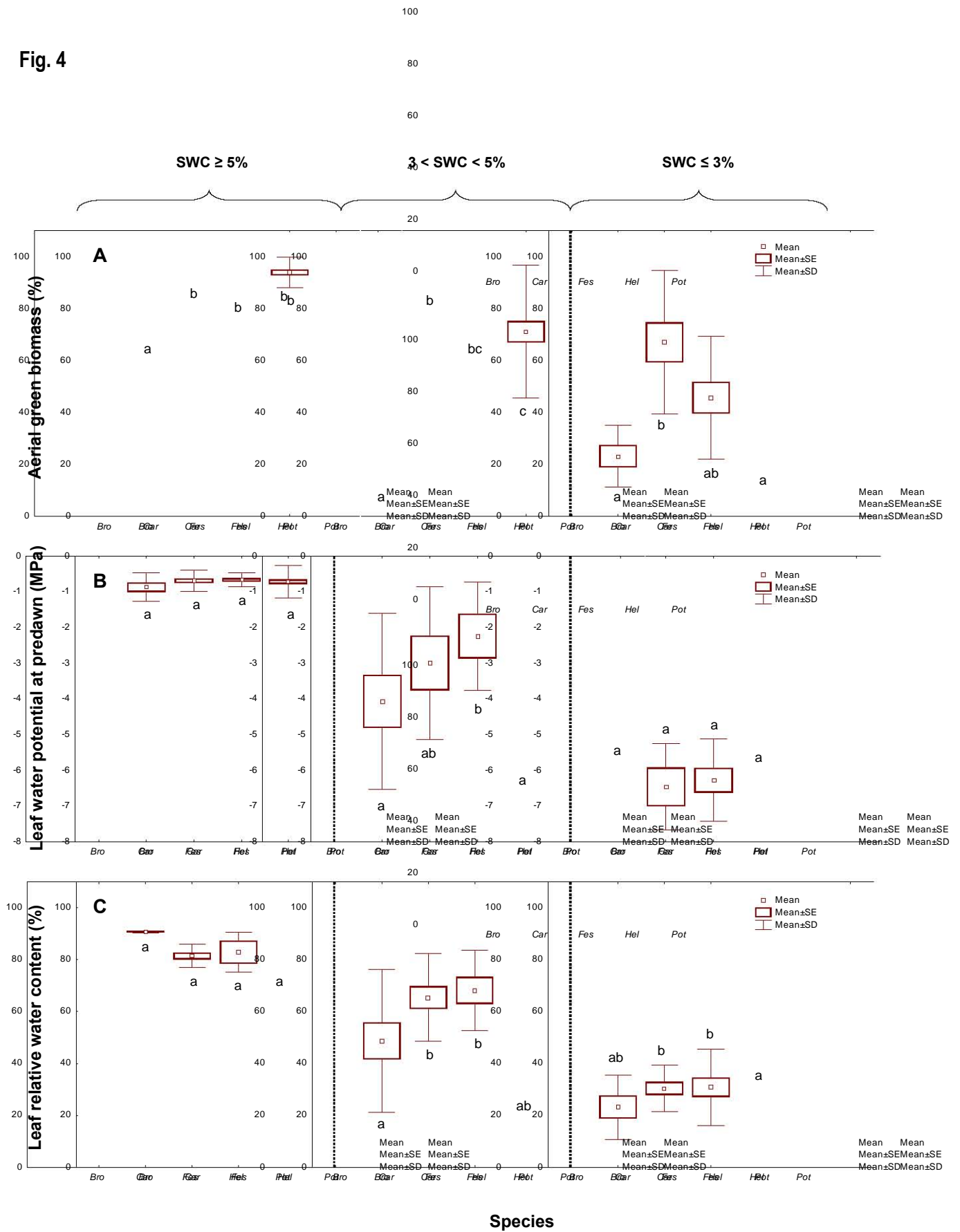


Fig. 5

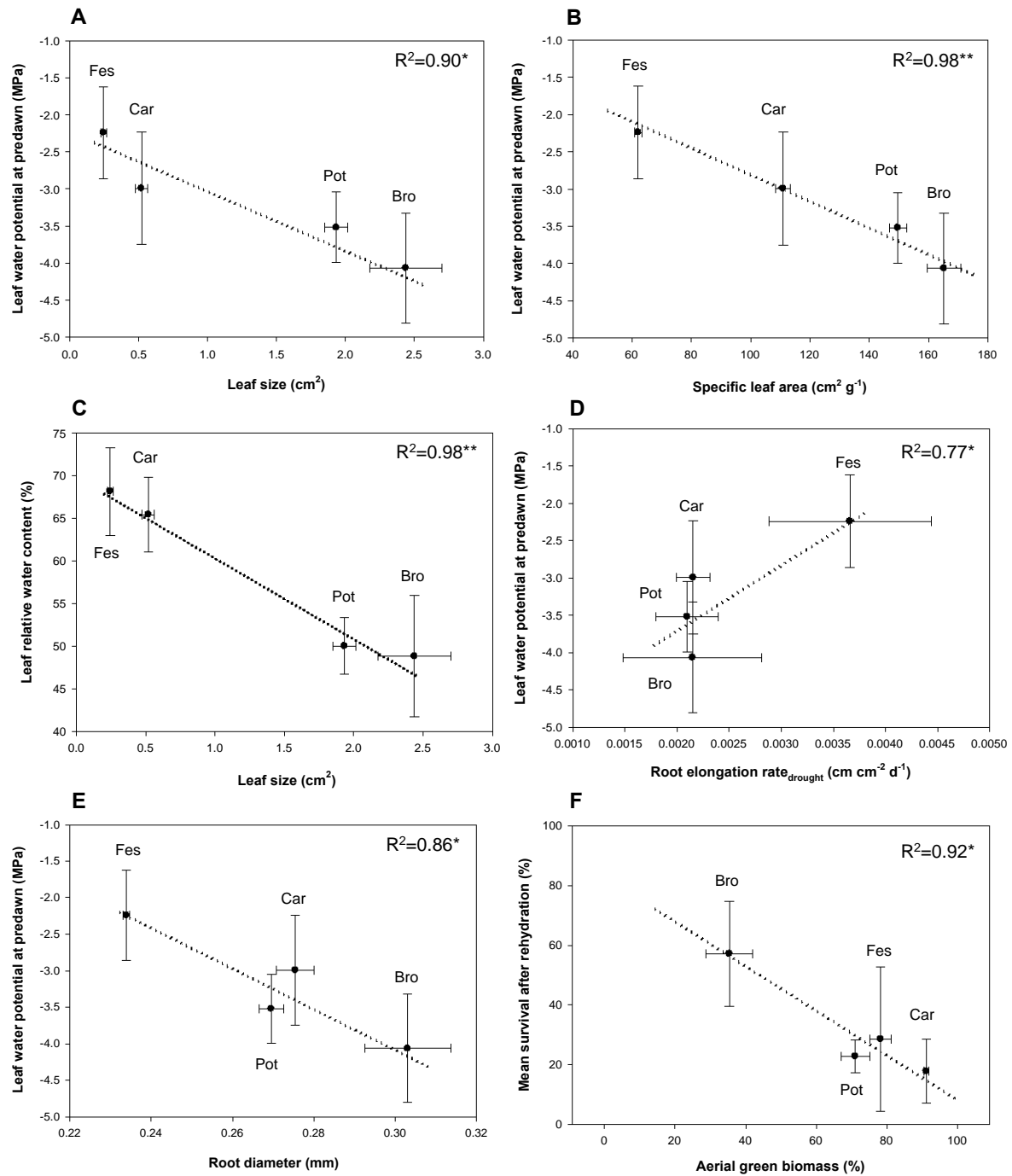


Fig. 6

