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1 **Original Article**

2

3 **Title:** What functional strategies drive drought survival and recovery of perennial species
4 from upland grassland?

5

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19 **Running title:** Drought strategies in perennial forage species

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21

1 **Abstract**

2 • **Backgrounds and aims.** Extreme climatic events such as severe droughts are
3 expected to increase with climate change and limit grassland perennity. We investigated the
4 plant strategies involved in drought survival and recovery of temperate herbaceous species.

5 • **Methods.** Monocultures of six native perennial species from upland grasslands and
6 one Mediterranean drought resistant cultivar were compared under semi-controlled and non-
7 limiting rooting depth conditions. Above- and below-ground traits were measured under
8 irrigation in spring and during drought in summer (50 days of withholding water) to
9 characterise resource use and drought resistance strategies. They were then rehydrated to
10 assess survival (15 days) and recovery (1 year).

11 • **Key results.** Dehydration avoidance through water uptake was associated with species
12 that had deep roots (> 1.2 m) and high root mass (> 4 kg m⁻³). Cell membrane stability
13 ensuring dehydration tolerance of roots and meristems was positively and negatively
14 correlated to fructan and sucrose contents, respectively. Species that survived and recovered
15 best combined high resource acquisition in spring (leaf elongation rate > 9 mm d⁻¹ and rooting
16 depth > 1.2 m) and both high dehydration avoidance and tolerance strategies.

17 • **Conclusion.** The variability of plant strategies observed in these species emphasizes
18 the role of functional diversity in sustaining the drought resilience of perennial grasslands.

19

20 **Key words:** dehydration avoidance, dehydration tolerance, drought survival, forbs, fructans,
21 grasses, membrane stability, resilience index, root system, sucrose

22

23

1 INTRODUCTION

2 In Europe, grasslands cover 22% of the EU-25 land area (FAOSTAT data,
3 <http://faostat.fao.org>) and provide various ecosystemic services such as forage production,
4 carbon storage, soil protection and biodiversity preservation (Millenium Ecosystem
5 Assessment, 2005). Climate change will likely impact this ecosystem through shifts in
6 grassland composition and production potential (Grime *et al.*, 2000; Gilgen and Buchmann,
7 2009; Craine *et al.*, 2011; Zwicke *et al.*, 2013). In temperate areas, more extreme rainfall
8 regimes are expected to increase the duration and severity of drought (Knapp *et al.*, 2008;
9 Seneviratne *et al.*, 2012). In this context, more knowledge is required to understand the
10 mechanisms of resistance, survival under severe drought and recovery of herbaceous species
11 after drought (Chaves *et al.*, 2003; Volaire *et al.*, 2014).

12 There is evidence for a persistence-productivity trade-off, species with a high maximum
13 growth rate having a higher mortality under restricted resources (Sibly and Calow, 1989;
14 Wright *et al.*, 2010). In the case of drought tolerance, evidence for this trade-off has been
15 described for woody plants (Reich, 2014), but the relationships between plant growth rate
16 under optimal conditions and subsequent performance under severe drought has been scantily
17 explored in herbaceous species (Pérez-Ramos *et al.*, 2013). Perennial herbaceous species are
18 interesting plant models, since they can resist and survive severe drought through adaptive
19 strategies contributing to dehydration avoidance and dehydration tolerance (Ludlow, 1989;
20 Blum, 1996). Under moderate drought, dehydration avoidance ensures the maintenance of
21 plant tissue hydration and osmotic potential by maximizing water uptake and minimizing
22 water losses (Ludlow, 1989; Volaire *et al.*, 2009). Under severe drought, once complete leaf
23 senescence is reached, dehydration tolerance ensures plant survival by the maintaining cell
24 integrity in meristematic tissues through cell membrane stabilization, accumulation of water
25 soluble carbohydrates (WSC) and dehydrins (Voltaire *et al.*, 1998a, b; Verslues *et al.*, 2006).

1 Grass species of temperate areas accumulate large amounts of reserve carbohydrates mainly
2 as fructans in leaf meristems (Chatterton *et al.*, 1989; Pollock and Cairns, 1991). It has been
3 shown that this accumulation during drought improves plant survival after drought (Volaire *et*
4 *al.*, 1998a; Clark *et al.*, 2004). Fructans in particular were shown to stabilize membranes in
5 *vitro* by interaction with lipids under stress (Vereyken *et al.*, 2001, 2003; Hinch *et al.*, 2002,
6 2007) and because they may act as antioxidants (Peshev *et al.*, 2013). These compounds play
7 an indirect role in drought survival when they are hydrolysed to fuel growth after rehydration
8 (Volaire *et al.*, 1998b; Thomas and James, 1999; Amiard *et al.*, 2003).

9 Under progressive soil drying, higher biomass allocation to roots (Poorter *et al.*, 2012) and
10 extensive root system have been commonly observed in drought resistant species (Comas *et*
11 *al.*, 2013), since deep roots can take up water from moister soil layers. Under severe soil
12 drought conditions root mortality increases due to tissue dehydration (Eissenstat and Yanai,
13 1997; Facette *et al.*, 1999). However, it has been shown in ryegrass that some roots can
14 survive very dry conditions (soil water content < -10 MPa), especially root apices, and that
15 production of lateral roots from existing roots is observed after soil rewetting (Jupp and
16 Newman, 1987). Thus maintaining live roots in severe soil drying conditions should enhance
17 plant recovery (Weaver and Zink, 1946; Huang *et al.*, 1997). However, despite of their crucial
18 role in water uptake under drought, below-ground plant traits remain poorly quantified
19 compared with leaf traits (Reich, 2014). The combination of these plant responses depends on
20 species and genotypes, and can be investigated by assessing leaf and root traits (Pérez-Ramos
21 *et al.*, 2013) associated with water status and WSC metabolism (Volaire, 2008).

22 The present study aimed (i) to characterize the adaptive responses whereby temperate
23 grassland species resist, survive and recover from a severe drought and (ii) to explore the
24 relationships between plant resource use and drought resistance strategies. To this end, we
25 compared six dominant perennial herbaceous species (five grasses and one forb) originating

1 from upland temperate grasslands (Louault *et al.*, 2005), according to their resistance to and
2 recovery from summer extreme (Zwicke *et al.*, 2013), with a Mediterranean grass cultivar
3 used as control for its high drought survival. These species were grown in semi-controlled and
4 non-limiting rooting depth conditions to analyse leaf and root traits. Optimum resource-use
5 strategies were identified under irrigation in spring, dehydration avoidance strategy was
6 assessed under moderate drought (20 days of withholding water) and dehydration tolerance
7 was estimated under severe drought (50 days). Plant survival and resilience indices (according
8 to Van Ruijven and Berendse, 2010) were respectively measured two weeks and one year
9 after rehydration. We tested the following hypotheses: (i) the drought survival of temperate
10 species mainly depends on dehydration avoidance through water acquisition strategies, (ii)
11 accumulation of WSC, especially fructans and sucrose in surviving organs including not only
12 leaf meristems, but also roots and root apices, is associated with dehydration tolerance, and
13 (iii) there is a trade-off between high resource-use under non-limiting conditions and high
14 ability to survive and recover from severe drought.

15

16 MATERIALS AND METHODS

17 *Experimental set-up and conditions*

18 The experiment was conducted outdoors at Clermont-Ferrand (45°47'N, 03°05'E, 350 m
19 a.s.l.), under a semi-continental climate (mean annual temperature, 12.4 °C; mean annual
20 precipitation, 579 mm). An automatic weather station recorded air temperature, global
21 radiation, wind speed and vapour pressure deficit, allowing the calculation of potential
22 evapotranspiration (PET) (Table S1).

23 In summer 2010, granitic brown soil (12% clay, 17% loam, 59% sand, 13% organic
24 matter), extracted from an upland grassland (45°43'N, 03°01'E, 850 m a.s.l.), was sieved at 7
25 mm and left to dry in the air. Plastic sleeves were placed inside 105 deep tubes (PVC, 150 cm
26 deep, 10 cm diameter) for easy sampling of the whole root system. The tubes were filled with
27 the soil mixed with slow-release fertiliser (3.5 kg m⁻³, NPK 14-7-14, Multicote 12, Haifa,

1 Israel). In September 2010, seeds of native species: *Dactylis glomerata* (*Dg*), *Festuca*
2 *arundinacea* (*Fa*), *Poa pratensis* (*Pp*), *Poa trivialis* (*Pt*), *Trisetum flavescens* (*Tf*) and
3 *Taraxacum officinale* (*To*) from an upland grassland (45°43'N, 3°01'E, 880 m a.s.l., Pontes *et*
4 *al.*, 2007) and seeds of a Mediterranean cultivar of *D. glomerata* 'Medly' (RAGT, France)
5 (*Md*), were sown at a density of 2500 seeds m⁻² in each tube. This Mediterranean cultivar was
6 used as a control for high drought survival (Volaire and Lelièvre, 2001). All the tubes were
7 placed outside in soil trenches during the winter and kept well-watered with rainfall and
8 additional watering.

9 In spring 2011 a drip irrigation system was set up (day of year, DOY 78) to maintain soil
10 water content (SWC) near field capacity. In addition, three volumetric SWC (m³ m⁻³) probes
11 (ECHO-5, Decagon, USA) were inserted horizontally at depths 10, 40 and 100 cm on three
12 tubes per species, and connected to a datalogger (EM50, Decagon, USA). From May to
13 November (DOY 132 to 326), SWC was measured every 15 min (Fig. 1). Three tubes per
14 species were also installed on a weighing scale (60 x 60 cm, Arpege Master K, type N PAC +
15 SAT MB, France) to record plant water-use (WU, g kg⁻¹) by gravimetry. To limit soil
16 warming due to light radiation, the tubes were insulated with polystyrene (50 mm thick,
17 Styrodur®, BASF, France).

18 The drought treatment (50 days of withholding water) was induced from DOY 182 by
19 stopping irrigation and intercepting all precipitations with a transparent polycarbonate shelter
20 (12.5 x 10.8 m, 6.2 m high, 90 % transmitted PAR, Batiroc, France). This shelter was
21 automatically controlled by a rain sensor. The drought treatment consisted first of 20 days of
22 gradual soil drying until cessation of leaf growth (moderate drought, DOY 182-201), and then
23 30 days at SWC < 0.1 m³ m⁻³ (severe drought, DOY 202-231) (Fig. 1). This treatment was
24 applied on 12 tubes per species. Three tubes per species were maintained under full irrigation
25 for the control treatment. From DOY 232, the tubes were rehydrated and maintained under

1 full irrigation until the growing season ended (DOY 306). All the tubes then received local
2 precipitation until the end of the experiment in June 2012 (DOY 174). Finally 21 and 84 tubes
3 were used respectively for control and drought treatments.

4 During the experiment, plants were cut to a height of 5 cm, six times in 2011 (DOY 97,
5 116, 136, 165, 230, 271) and then twice until spring 2012 (DOY 78, 151).

6

7 *Leaf and root traits*

8 Leaf length (mm) of 36 mature tillers per species was measured every 2–3 days from DOY
9 146 to 193 (moderate drought conditions) to calculate leaf elongation rate (LER, mm d⁻¹,
10 Carrère *et al.*, 1997). During the drought treatment (DOY 193-222), leaf senescence (LS, 0-
11 100 %) was visually assessed every 2-5 days.

12 Three tubes per species were harvested at four sampling dates (SD), corresponding to the
13 end of the watering period (DOY 180, SD1), the end of moderate drought after the cessation
14 of leaf growth (DOY 200, SD2), the end of severe drought (DOY 231, SD3) and to the
15 control treatment (DOY 242, SD4) in which plants were not subjected to drought (controls).

16 For each SD, nine mature leaves per species were collected and rehydrated to measure leaf
17 relative water content (LRWC, %) according to Volaire *et al.* (1998b). Specific leaf area
18 (SLA, m² kg⁻¹) and leaf dry matter content (LDMC, g g⁻¹) were measured at SD1. Leaf
19 meristems (enclosed leaf bases) were sampled after removal of mature leaves (Lattanzi *et al.*,
20 2004), and fresh and dry (oven-dried at 60°C for 48h) weighed to determine their water
21 content (MWC, %). For *P. trivialis* and *T. flavescens*, stolons (storage organs) were also
22 collected for subsequent WSC measurements. The whole intact root system was extracted
23 from each tube and carefully washed with tap water. Maximum rooting depth was measured
24 (Dmax, cm), then deepest root apical zone (15 cm length, called root apices) and other roots
25 were collected according to five soil layers (0-15, 15-30, 30-40, 40-90, and 90-150 cm,

1 hereafter called roots). For *P. pratensis* and *T. officinale*, rhizomes and tap root (storage
2 organs) were separated from fine roots. Root dry matter content (RDMC, g g⁻¹) and total root
3 mass (RM, kg m⁻³) were calculated. The 95% rooting depth (D₉₅, cm), i.e. the depth including
4 95% of the root biomass, was calculated according to Schenk and Jackson (2002).

5

6 *Cell membrane stability (CMS)*

7 At the end of severe drought (SD3, SD4), three sub-samples of leaf meristems, roots from
8 each soil layer, root apices and storage organs were harvested to measure CMS (%),
9 according to Volaire (1995) and Charrier and Améglio (2011). The CMS of each root layer
10 was averaged (CMSr).

11

12 *Water soluble carbohydrate (WSC) analysis*

13 At each SD, fresh sub-samples of leaf meristems, roots per soil layer, root apices and storage
14 organs (rhizomes, stolons, and tap roots) were quickly fresh-weighed, dropped into liquid
15 nitrogen and stored at -80 °C before freeze-drying (-100 °C for 48 h). Fine powder samples
16 (30-50 mg) were extracted in 80 % ethanol, and purified in mini-columns (Mobicols from
17 MoBITec, Göttingen, Germany) with ion-exchange resins (see Amiard *et al.*, 2003 for
18 details). Water soluble carbohydrates (mg g⁻¹) were analysed by high performance liquid
19 chromatography (HPLC) on a cation exchange column (Sugar-PAK, 300 X 6.5 mm, Millipore
20 Waters, Milford, MA, USA) eluted at 0.5 mL min⁻¹ and 85 °C with 0.1 mM Ca-EDTA in
21 water and quantified using a refractive index detector (2410 Differential Refractometer,
22 Millipore Waters). External standards used to quantify carbohydrates were glucose, fructose,
23 sucrose and *Cichorium intybus* inulin (Sigma-Aldrich, MO, USA). The HPLC system enabled
24 us to separately quantify fructans with degrees of polymerization 3 and 4 (low degree of
25 polymerization, LDP) and over 5 (high degree of polymerization, HDP). The mean WSC

1 content of the whole root system was calculated from the WSC content (mg g^{-1} DW) and the
2 dry mass of each root layer. In addition, amount of WSCs (mg per plant) in roots (QWSCr)
3 was calculated by multiplying dry mass of roots (g per plant) with WSC content (mg g^{-1} DW)
4 measured after severe drought.

5

6 *Plant survival, recovery and resilience indices*

7 Two weeks after rehydration (DOY 246) and in the following spring 2012 (DOY 158),
8 percentage of green tissue in aerial tissues was visually assessed on three tubes per species to
9 determine plant survival (SURV) and one-year recovery (RECOV).

10 In spring 2011 (DOY 97, 116, 136, 165) and spring 2012 (DOY 78, 151) above-ground
11 biomass was oven-dried ($60\text{ }^{\circ}\text{C}$ for 48 h) and weighed to determine pre- and post-drought
12 forage production. In addition, three tubes per species were harvested in spring 2011 (SD1)
13 and in spring 2012 (DOY 172) to assess pre- and post-drought standing root mass (kg m^{-3}).
14 Resilience indices of spring forage production (RI) and spring standing root mass (RRI) were
15 calculated as the ratio of post-drought (2012) to pre-drought (2011) biomass and standing root
16 mass, respectively (Van Ruijven and Berendse, 2010).

17

18 *Statistical analyses*

19 Statistical analyses were carried out with R software (R Core Team, 2012). Data were
20 transformed when necessary (arcsine-square root-transformed, square root) before analysis to
21 conform to the assumptions of normality (Shapiro-Wilk test) and homogeneity of variances
22 (Fligner Killeen test). For each SD, single factor (species, treatment) variance analysis
23 (ANOVA) and the *post hoc* Tukey test were performed with the ‘multcomp’ package
24 (Hothorn and Bretz, 2009). Soil water content was estimated at the end of leaf growth
25 (SWC_{el}) and for 50% of leaf senescence (SWC_{s}) from linear and sigmoid regressions between

1 LER with SWC and LS with SWC, respectively. In addition, a Student test was used to
2 compare means of two variables (green tissue percentage, resilience indices) for each species.
3 Pearson's coefficients of correlation between plant traits were calculated with the 'Hmisc'
4 package (Harrel and Dupont, 2007). Plant functional strategies for resource acquisition,
5 dehydration avoidance and dehydration tolerance were analysed with matrices crossing traits
6 and species replicates, using three principal component analyses (PCA) with the 'ade4'
7 package (Dray and Dufour, 2007). The first PCA tested seven traits measured before drought.
8 The second PCA tested six traits measured during the moderate drought. The third PCA tested
9 10 traits measured during moderate or severe drought. For each PCA, SURV, RECOV, RI
10 and RRI were added as supplementary variables. Multiple regressions with backward
11 selection then identified the traits most closely correlated with plant performance.

12

13 RESULTS

14 *Soil volumetric water content*

15 Before drought, SWC in shallow soil layers (depths 10 cm and 40 cm) fluctuated according to
16 irrigation or rainfall events and plant transpiration, and averaging $0.139 \text{ m}^3 \text{ m}^{-3}$ across species.
17 At depth 100 cm, SWC varied less and averaged $0.250 \text{ m}^3 \text{ m}^{-3}$ (Fig. 1). One week after the
18 last irrigation, SWC in shallow soil layers rapidly decreased and stabilized at $0.045 \text{ m}^3 \text{ m}^{-3}$. At
19 100 cm depth and during the moderate drought, SWC declined to either $0.102 \text{ m}^3 \text{ m}^{-3}$ (*F.*
20 *arundinacea*, *T. officinale*, *D. glomerata* 'Medly') or $0.279 \text{ m}^3 \text{ m}^{-3}$ (*T. flavescens*, *P. pratensis*
21 and *P. trivialis*), and remained stable during the severe drought. After rehydration (DOY 232-
22 246), SWC in shallow and deep layers reached 0.174 and $0.295 \text{ m}^3 \text{ m}^{-3}$, respectively.

23

24

25

1 *Plant traits before drought*

2 All traits differed significantly between species (Table 1). Specific leaf area (SLA) ranged
3 between 49.5 (*P. trivialis*) and 22.8 (*P. pratensis*) m² kg⁻¹. *D. glomerata*, *D. glomerata*
4 ‘Medly’ and *T. officinale* had the highest elongation rate (LER > 9 mm d⁻¹), while *T.*
5 *flavescens*, *F. arundinacea*, *P. trivialis* and *P. pratensis* had the lowest (LER < 7 mm d⁻¹).
6 Total root mass (RM₁, P < 0.001) discriminated *T. officinale* and grasses due to the high tap
7 root mass (7.5 kg m⁻³). Within grasses, *F. arundinacea*, *D. glomerata* and *D. glomerata*
8 ‘Medly’ had the highest RM₁ and *P. trivialis* the lowest. Higher values of maximum rooting
9 depth (Dmax₁) and 95% rooting depth (D95₁) were observed for *T. officinale*, *F. arundinacea*,
10 *D. glomerata* and *D. glomerata* ‘Medly’, whereas *P. trivialis* had the shortest root system (P <
11 0.001, Fig. 2). Finally, water used (WU₁) was significantly higher for *T. officinale* and *F.*
12 *arundinacea* (> 120 g kg⁻¹, P < 0.001) than for species with shallower root systems, such as *T.*
13 *flavescens*, *P. pratensis* and *P. trivialis*. Root dry matter content (RDMC₁) ranged between
14 0.101 (*To* fine roots) to 0.228 g g⁻¹ (*P. pratensis*), *D. glomerata* showing significantly lower
15 values (0.189 g g⁻¹) than *D. glomerata* ‘Medly’ (0.211 g g⁻¹, P < 0.01).

16 For all species and all analysed tissues, fructans with a high degree of polymerization (DP
17 ≥ 5, HDP fructans) were the dominant sugar compounds (73.2 ± 1.6 %) of total water soluble
18 carbohydrates (WSC), followed by sucrose (17.8 ± 1.2 %), fructans with a low degree of
19 polymerization (DP = 3-4, LDP fructans, 7.1 ± 0.4 %) and monosaccharides (glucose and
20 fructose, 4.4 ± 0.6 %). The present study focuses on the dominant carbohydrates (HDP
21 fructans and sucrose). Results for the other sugars (LDP fructans and monosaccharides) are
22 presented as supplemental data [Fig. S1, S2]. Before drought, HDP fructan contents in leaf
23 meristems and root apices were not significantly different between species (Fig. 3A, 3E). By
24 contrast, species were discriminated by HDP fructans and sucrose contents in roots (P <
25 0.001) to form three groups: (i) *T. officinale* and *T. flavescens*, (ii) *P. pratensis*, (iii) *D.*

1 *glomerata*, *D. glomerata* ‘Medly’, *F. arundinacea* and *P. trivialis* (Fig. 3C, 3D). Three
2 species (*T. officinale*, *P. pratensis*, and *P. trivialis*) accumulated fructans and sucrose in
3 storage organs (Fig. 3G, 3H).

4

5 *Plant responses to moderate drought*

6 For all the species, LER rapidly declined and stopped 10-12 days after the last irrigation when
7 SWC_{el} reached on average $0.090 \text{ m}^3 \text{ m}^{-3}$ and $LS_2 = 23\%$, respectively (Table 1). Leaf relative
8 water content ($LRWC_2$), meristem water content (MWC_2) and WU_2 decreased ($P < 0.001$),
9 $RDMC_2$ and LS_2 increased ($P < 0.001$) in comparison with irrigated treatment, whereas RM_2 ,
10 $Dmax_2$, and $D95_2$ did not change significantly. However all trait responses to moderate
11 drought differed between species (Table 2). The forb species (*T. officinale*) showed higher
12 values of $LRWC_2$ and MWC_2 and lower values of $RDMC_2$ than the grass species. Within
13 grasses, $LRWC_2$ and MWC_2 values were higher than 33% and 46%, respectively, for *D.*
14 *glomerata*, *F. arundinacea*, *D. glomerata* ‘Medly’ and lower for the remaining species (Table
15 1). Leaf senescence (LS_2) was the highest for *P. trivialis*, $RDMC_2$ of *T. flavescens* was
16 significantly lower than that of *D. glomerata* ‘Medly’ and *F. arundinacea*. For RM_2 , $Dmax_2$
17 and $D95_2$, between-species differences followed the same pattern as observed before drought
18 (Fig. 2). Water used (WU_2) of *T. officinale* was about twice higher than that of *T. flavescens*, *P.*
19 *pratensis* and *P. trivialis* (Table 1).

20 Across species HDP fructan contents did not change in response to the moderate drought in
21 leaf meristems and in root apices (Fig. 3A, 3E, Table 2), whereas sucrose contents increased
22 in all organs, especially in leaf meristems (+177 %, $P < 0.001$) and root apices (+72 %, $P <$
23 0.01) (Fig. 3B, 3F, Table 2). The pattern of HDP fructans in roots and the pattern of sucrose
24 in leaf meristems, roots and root apices differed between species. For *T. officinale*, HDP
25 fructan and sucrose contents in roots and taproot were maintained whereas significant

1 increases in HDP fructans (+61%) and sucrose (+73%) contents were observed in roots of *D.*
2 *glomerata* ‘Medly’ (Fig. 3C, 3D, $P < 0.001$). Sucrose content in leaf meristems remained
3 stable for *D. glomerata* ‘Medly’ and *D. glomerata*, but strongly increased in *T. flavescens*, *P.*
4 *pratensis*, *F. arundinacea* and *P. trivialis* (+200 % on average, Fig. 3B, $P < 0.01$). An
5 accumulation of sucrose was also observed in roots of *D. glomerata* and *F. arundinacea* (+92
6 % and +56 %, respectively, $P < 0.01$), and in root apices of *D. glomerata* (+362 %, $P < 0.05$).
7 Conversely for *T. flavescens* and *P. trivialis*, HDP fructan content in roots decreased (-52 %
8 on average, $P < 0.01$), whereas sucrose content in roots and root apices remained stable (Fig.
9 3B, 3C, 3D). Also, sucrose content increased in stolons of *P. trivialis* (+219 %, $P < 0.001$) but
10 did not change significantly in rhizomes of *P. pratensis* (Fig. 3H). Content of fructans with a
11 low degree of polymerization (3-4, LDP fructans) decreased in root apices (-71 %, Fig. S1F).

12 At the end of moderate drought, HDP fructan content in leaf meristems was more than
13 twice as high in *D. glomerata* ‘Medly’ as in the upland species, whereas sucrose content was
14 much higher in *T. flavescens*, *P. pratensis* and *F. arundinacea* than in *T. officinale*, *D.*
15 *glomerata* ‘Medly’ and *D. glomerata* (Fig. 3A, 3B). In roots, HDP fructan content of *T.*
16 *officinale*, *P. pratensis*, *T. flavescens* and *D. glomerata* ‘Medly’ was higher than that of *D.*
17 *glomerata*, *F. arundinacea*, *P. trivialis* (Fig. 3C). Roots of *P. pratensis* showed the highest
18 sucrose content, 130 % higher than in roots of *P. trivialis* (Fig. 3D). *T. flavescens* had eight
19 times higher HDP fructan content in root apices than *D. glomerata*, *F. arundinacea*, and *P.*
20 *trivialis* (Fig. 3E). Regarding the storage organs, taproot of *T. officinale* had the highest HDP
21 fructan content, whereas sucrose content was higher in the rhizomes of *P. pratensis* (Fig. 3G,
22 3H).

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24
25

1 *Plant responses to severe drought*

2 For all the species, leaf senescence (LS₃) increased, reaching on average 93 % (P < 0.001,
3 Table 1). Leaf relative water content (LRWC₃) of the remaining living leaves and MWC₃
4 declined on average to 18 % and 31 %, respectively (P < 0.001), and WU₃ was half that under
5 the moderate drought (P < 0.001). Root dry matter content (RDMC₃) increased significantly
6 (P < 0.001) whereas RM₃ and Dmax₃ were unchanged between SD₂ and SD₃. Contents of
7 HDP fructan and sucrose in leaf meristems were not affected by the severe drought (Table 2).
8 However, in roots, HDP fructan content significantly decreased by 49 % on average for *T.*
9 *officinale*, *D. glomerata* ‘Medly’ and *P. pratensis* (P < 0.01), and sucrose content also
10 declined for *T. officinale*, *D. glomerata* ‘Medly’, *D. glomerata*, *F. arundinacea* and *P.*
11 *pratensis* (P < 0.01, Fig. 3C, 3D). Production of stolons appeared in *T. flavescens* during the
12 severe drought, and this organ accumulated HDP fructan and sucrose contents similar to those
13 of *P. trivialis* (Fig 3G, 3H).

14 Cell membrane stability (CMS) significantly declined in response to the drought (P <
15 0.01), except in leaf meristems of *D. glomerata* ‘Medly’ and *T. flavescens*, in root apices of
16 *D. glomerata* ‘Medly’ and *P. trivialis*, and in stolons of *T. flavescens* (Fig. 4). Considering
17 both treatments and all the species together, CMS of leaf meristems, roots and storage organs
18 was positively correlated to the HDP fructan content of the corresponding tissues ($r^2 = 0.16$, P
19 < 0.01; $r^2 = 0.31$, P < 0.001; $r^2 = 0.10$, P < 0.01, respectively), measured after severe drought
20 in stressed and control plants (Fig. 5A, 5C, 5E). By contrast, CMS was negatively correlated
21 to sucrose content ($r^2 = 0.24$, P < 0.01 in leaf meristems and $r^2 = 0.24$, P < 0.01 in storage
22 organs). At species level, correlation between CMS and HDP fructans could not be found in
23 leaf meristems but in roots CMS and HDP fructan content were positively linked ($r^2 > 0.39$; P
24 < 0.001) for each species except *P. trivialis* (Fig. 5C). Cell membrane stability was negatively
25 correlated to sucrose content in leaf meristems of *F. arundinacea* and *T. officinale*, in roots of

1 *D. glomerata*, *D. glomerata* ‘Medly’, *P. pratensis* and *T. flavescens*, and in the storage organs
2 of the four species considered (Fig. 5B, 5D, 5F). Cell membrane stability was not correlated
3 with carbohydrate contents in root apices (data not shown).

4

5 *Plant survival and one-year recovery after drought*

6 All the species were able to regrow two weeks after rehydration, but the percentage of green
7 tissue in aerial tissue (SURV) differed significantly between species ($P < 0.001$, Fig. 6A, 6B).
8 Thus, three groups of species were identified: (i) high survival for *T. officinale* and *D.*
9 *glomerata* ‘Medly’ (green tissue $> 90\%$), (ii) intermediate survival for *D. glomerata*, *T.*
10 *flavescens* and *P. pratensis* (50-70%), and (iii) low survival for *F. arundinacea* and *P.*
11 *trivialis* ($< 20\%$). However, greenness measured one-year after drought application
12 (RECOV) was not significantly different between species, but high standard error values were
13 noted for *T. flavescens*, *F. arundinacea* and *P. trivialis* (Fig. 6B).

14 Resilience index of spring forage production (RI) was significantly higher than that for *D.*
15 *glomerata* ‘Medly’ ($P < 0.01$), and large between-species differences were observed ($P <$
16 0.001 , Fig. 6C). *D. glomerata* ‘Medly’, *P. pratensis* and *F. arundinacea* showed the highest
17 RI and *T. officinale*, *T. flavescens* and *P. trivialis* the lowest. Resilience index of spring
18 standing root mass (RRI) was close to one, except for *D. glomerata* ‘Medly’ and *D.*
19 *glomerata* ($P < 0.05$), which showed the highest values.

20

21 *Plant strategies during drought and after rehydration*

22 Pearson’s correlation coefficients (Table 3) showed positive and significant correlations
23 between plant survival (SURV) and some of the traits measured before drought (LER, RM_1 ,
24 $Dmax_1$), after moderate drought (HDP_r_2 , $LRWC_2$, RM_2 , SWC_{el} , WU_2) and during severe
25 drought (MWC_3 , $CMSm$ and SOr_3). SURV was negatively correlated with LS_3 , $RDMC_2$, and

1 SOM₂. The resilience index of spring forage production (RI), was positively correlated with
2 traits measured before drought (Dmax₁, D95₁ and RDMC₁), and also during severe drought
3 (CMSr, QWSCr and SOa₃). The resilience index of spring forage production was also
4 negatively correlated to SLA and SWCs. However, only positive correlation with SWCel was
5 observed for RECOV and RRI, which were correlated with each other and not with SURV.

6 The first two axes of the PCA performed with traits related to resource acquisition before
7 drought (Fig. 7A), dehydration avoidance (Fig. 7B) and dehydration tolerance during drought
8 treatment (Fig. 7C) accounted for 75.9%, 91.1% and 54.7% of the total variance observed
9 among species, respectively. For each PCA, SURV was closely correlated to the first axis:
10 0.65, -0.74 and -0.73, respectively. The resilience index of spring forage production was
11 correlated to the second axis of the PCA related to resource acquisition (0.74) and dehydration
12 avoidance (0.66). However, RECOV and RRI were not explained by these syndromes of
13 traits. The multiple regressions performed with these traits showed that the contribution to
14 SURV differed according to the strategies with the following decreasing ranking: dehydration
15 tolerance strategy ($r^2 = 0.76$, $P < 0.001$), dehydration avoidance ($r^2 = 0.54$, $P < 0.001$) and
16 resource acquisition before drought ($r^2 = 0.44$, $P < 0.001$). Conversely, RI was mostly
17 associated with the resource acquisition strategy ($r^2 = 0.68$, $P < 0.001$).

18 The first axis of PCAs, related to resource acquisition (Fig. 7A), and drought avoidance
19 (Fig. 7B) showed a main contrast between the species with small root systems (*P. trivialis*, *P.*
20 *pratensis* and *T. flavescens*) and those with deep ones (*D. glomerata*, *F. arundinacea*, *D.*
21 *glomerata* ‘Medly’ and *T. officinale*) (Fig. 7A, 7B, $P < 0.001$). Species were also segregated
22 by the first axis of the PCA related to dehydration avoidance with a main contrast between the
23 forb (*T. officinale*) and the grass species (Fig. 7B, $P < 0.001$), while the first axis of the PCA
24 related to tolerance strategy mostly opposed the upland species from the Mediterranean
25 cultivar of *D. glomerata* (Fig. 7C, $P < 0.001$).

1 When considering only the six species from the upland grassland, the multiple regressions
2 show a modified contribution of traits of both dehydration avoidance ($r^2 = 0.65$, $P < 0.01$), and
3 dehydration tolerance after backward selection (HDPr, SOm, MWC₃ and CMSm, $r^2 = 0.67$, P
4 < 0.001) on drought survival.

5

6 DISCUSSION

7 *Temperate and Mediterranean species display different strategies for dehydration avoidance*

8 Against our first hypothesis, the results show a similar contribution of dehydration avoidance
9 and tolerance to drought survival in species from temperate upland grassland. Dehydration
10 avoidance is achieved through a combination of responses favouring maximized water uptake
11 and minimized water loss under drought (Ludlow, 1989). The changes in relative water
12 content in tissues (MWC and LRWC, Table 1) are indicators of the cellular adjustments
13 (accumulation of solutes and/or cell wall hardening) made by the plant to achieve a low water
14 potential while avoiding water loss (Verslues *et al.*, 2006). In this study, LRWC at moderate
15 drought was correlated to RM, WU and plant survival (Fig. 7B, Table 3), which confirms that
16 maximizing water uptake during drought is an important mechanism in maintaining the
17 hydration of lamina (Passioura, 1981; Chaves and Pereira, 1992; Volaire *et al.*, 2009; Lelièvre
18 *et al.*, 2011). As previously shown for other species (Jackson *et al.*, 2000; Pinheiro *et al.*,
19 2005; Volaire, 2008), water uptake is mainly associated with initial root traits (Table 3). The
20 species with higher RM and D_{\max} , such as *T. officinale*, *D. glomerata* ‘Medly’, *D. glomerata*
21 and *F. arundinacea*, had a higher water-use before and during moderate drought (Table 1,
22 Table 3, Fig. 7B). In arid and semi-arid environments, relatively small proportions of roots in
23 deeper, moister soil layers may suffice to sustain water absorption of plants (Ehleringer and
24 Dawson, 1992). The maintenance of water uptake by roots during a drought period is mainly
25 driven by hydraulic continuity between soil, roots and leaves, which all depend on plant

1 transpiration, hydraulic properties of roots (Passioura, 1988) and also on root morphology and
2 anatomy (North and Nobel, 1991; Rieger and Litvin, 1999; Huang and Eissenstat, 2000;
3 Steudle, 2000; Hernández *et al.*, 2010). Between-species differences observed on leaf and root
4 traits such as LRWC, MWC and RDMC (Table 1) suggest differences in water acquisition
5 strategy, and therefore in the ability to maintain water uptake, explained by rooting depth
6 (Fig. 7A, 7B; Table 3) and differences in root diameter and tissue density (Picon-Cochard *et*
7 *al.*, 2012). Overall, root lifespan maintenance during drought could be related to root
8 hydration maintenance by the hydraulic lift mechanism (Bauerle *et al.*, 2008), cell osmotic
9 increase in cortex cells (Sharps and Davies, 1979), and suberization of the endoderm
10 protecting xylem cells to dehydration, which could explain at least part of the increase in
11 RDMC and decrease in plant water content (Table 1). The species able to maintain a root
12 production during moderate drought, such as *T. officinale* and *D. glomerata* ‘Medly’ (Table 1,
13 Fig. 2) had higher drought survival rates (Fig. 6B). Root growth maintenance under moderate
14 water stress was also identified as an important mechanism to avoid dehydration by
15 maximizing water uptake through an increase in root absorption area (Passioura, 1981, 1988;
16 Vartanian, 1981; Pérez-Ramos *et al.*, 2013).

17 Other mechanisms, such as modifications in water-use efficiency and regulation of
18 stomatal conductance are involved in dehydration avoidance (Jones *et al.*, 1981; Chaves and
19 Pereira, 1992; Golluscio and Oesterheld, 2007). In addition, when stress intensifies, increased
20 leaf senescence contributes to the minimization of water loss by reducing leaf area and
21 evaporation (Ludlow, 1989; Gepstein, 2004). In this study, leaf senescence, which was
22 inversely correlated to plant survival, was not adaptive but an indicator of drought
23 vulnerability (Fig. 7C, Table 3). Conversely, gradual foliage senescence was positively
24 correlated with plant survival in a range of Mediterranean grasses (Volaire *et al.*, 1998b;
25 Volaire and Lelièvre, 2001; Pérez-Ramos *et al.*, 2013). This suggests differences in plant

1 strategies for dehydration avoidance between temperate and Mediterranean herbaceous plants,
2 which can exhibit summer dormancy with induced foliage senescence, as a specific strategy
3 to survive extreme drought (Volaire and Norton, 2006).

4

5 *Water soluble carbohydrate metabolism is involved in dehydration avoidance and in*
6 *dehydration tolerance*

7 This study shows that plant survival is associated with high leaf meristem hydration and cell
8 membrane stability under severe drought (Table 3, Fig. 4), which confirms that the ability of
9 plant to protect leaf meristems is a key mechanism of dehydration resistance (including both
10 avoidance and tolerance strategies) to ensure plant survival (Volaire *et al.*, 1998b; Volaire and
11 Lelièvre, 2001). Higher dehydration avoidance and dehydration tolerance were both
12 previously attributed to WSC, through their involvement in osmotic adjustment and cell
13 membrane stabilization (Thomas, 1991; Volaire *et al.*, 1998b; Livingston *et al.*, 2009).
14 Interestingly, among non-specific storage organs, it was in the leaf meristems of all the
15 species that total WSC content was highest at the end of severe drought, but it was not
16 discriminating between species [Fig. S1A]. However, each carbohydrate was modulated
17 differently by drought according to the species considered (Fig. 3, Fig. S1, Fig. S2). This
18 indicates that carbohydrate composition rather than carbohydrate content is involved in
19 drought resistance mechanisms as already suggested (Ingram and Bartels, 1996).

20 When the photosynthetic machinery is impaired by prolonged drought (Chaves and
21 Pereira, 1992), carbohydrate contents are modified, which may act as metabolic signals to
22 promote leaf senescence and reserve mobilization (Zeppel *et al.*, 2011; McDowell, 2011; Sala
23 *et al.*, 2012). In the sensitive species, *T. flavescens*, *P. pratensis*, *F. arundinacea* and *P.*
24 *trivialis* (Fig. 6A, 6B), sucrose was accumulated in leaf meristems during the moderate
25 drought (Fig. 3B), and HDP fructan content declined significantly in the most sensitive ones,

1 *F. arundinacea* and *P. trivialis* (Fig. 3A). Accumulation of sucrose might result from sucrose
2 synthesis enhancement and/or from fructan degradation. Although increasing sucrose
3 synthesis could be stimulated by drought (Ingram and Bartels, 1996), more studies on enzyme
4 activities are needed to elucidate the role of fructan hydrolysis in sucrose accumulation under
5 drought. Hydrolysis of the polymers and concomitant synthesis of sucrose could be the
6 mechanism whereby plant tissues survive water deficits (Spollen and Nelson, 1994; Xue *et al.*,
7 2008; Saeedipour and Moradi, 2011). As sucrose is known to play an important role in
8 osmotic adjustment during drought (Jones *et al.*, 1981; Thomas, 1991; Volaire *et al.*,
9 1998a,b), these results suggest that sucrose is involved in the dehydration avoidance strategy
10 for the most sensitive populations. Depending on species and duration of drought, fructans are
11 either accumulated (Volaire and Lelièvre, 1997; Volaire *et al.*, 1998b; De Roover *et al.*,
12 2000), modified in chain length (Thomas, 1991; Volaire *et al.*, 1998b; Thomas and James,
13 1999), or reduced (Thomas, 1991; Spollen and Nelson, 1994; Clark *et al.*, 2004). In this study,
14 maintenance of HDP fructan content in leaf meristems during drought (Fig. 3A) could
15 contribute to higher survival in the more tolerant species, *T. officinale*, *D. glomerata* ‘Medly’
16 and *D. glomerata* (Fig. 6), as already reported for Mediterranean cultivar (Volaire and
17 Thomas, 1995; Volaire *et al.*, 1998b; Volaire, 2008).

18 Despite a decline in CMS, this comparative study clearly reveals a positive correlation
19 between HDP fructan content and CMS in roots for all the species, except for the most
20 sensitive one, *P. trivialis* (Fig. 5C). This can be attributed to the role of fructans in cell
21 membrane protection (Valluru and Van den Ende, 2008; Livingston *et al.*, 2009). While
22 several studies have demonstrated this role through *in vitro* approaches (Vereyken *et al.*,
23 2001; Hinch *et al.*, 2002, 2007), only one study on *D. glomerata* supported it *in planta*
24 (Volaire *et al.*, 1998b). Present data corroborate the protective role of fructans in *D.*
25 *glomerata* and support it in a broader panel of temperate species, including forbs and grasses.

1 In leaves of resurrection plants which do not accumulate fructans, sucrose is the main
2 carbohydrate contributing to drought tolerance by membrane and protein stabilization (Ingram
3 and Bartels, 1996; Scott, 2000). In fructan-accumulating plants, fructans might play this role.
4 In addition, we found that CMS of roots was not significantly correlated to plant survival
5 (Table 3). This suggests that dehydration tolerance of the root system was not enough to
6 ensure plant survival, underlining the role of leaf meristems in re-growth capacity after
7 rehydration in species from upland grasslands and especially in the Mediterranean genotype.

8

9 *The combination of resource acquisition, dehydration avoidance and tolerance strategies*
10 *enhance drought survival and recovery of temperate species*

11 As in Ludlow (1989), present results show that species were able to combine strategies for
12 resource acquisition and strategies for drought resistance (Fig. 7, Table 3). The highest
13 drought survival and recovery (RI index) was observed for the fast-growing species with
14 higher LER and deeper roots, such as *T. officinale*, *D. glomerata* ‘Medly’ and *D. glomerata*
15 (Table 1, Fig. 6, Fig. 7, Table 3). A higher LER or SLA can confer a competitive advantage
16 for maintaining photosynthesis and root growth, although the resulting greater leaf area may
17 be less efficient in avoiding dehydration (Pérez-Ramos *et al.*, 2013). As LER and WU were
18 positively correlated (Fig. 7A, Table 3), our results confirm that differences among species in
19 dehydration avoidance strategy are closely associated with strategy to acquire water
20 depending on root mass and depth (Chaves, 2002).

21 However, a dehydration avoidance strategy mainly based on maximizing water acquisition
22 was not efficient enough to ensure plant survival and fast recovery in the case of *F.*
23 *arundinacea*. This species had a deep root system and a high root mass, but was unable to
24 maintain LRWC and MWC, and therefore had a low survival rate (Table 1, Fig. 6B). This
25 could be explained by higher leaf transpiration and lower water-use efficiency in comparison

1 with *T. officinale*, *D. glomerata* ‘Medly’ and *D. glomerata* (Brock and Galen, 2005; Milbau *et*
2 *al.*, 2005). However, at a longer time scale, *F. arundinacea* was able to recover to the level of
3 *D. glomerata* ‘Medly’, *D. glomerata* and *T. officinale*. In addition, species that were least
4 efficient in avoiding dehydration, but which had storage organs, such as *T. flavescens* and *P.*
5 *pratensis*, had higher drought survival than *F. arundinacea* (Fig. 5B), probably due to the
6 amount of carbohydrate reserves in their storage organs (Fig. 3G, 3H, Fig. S1G). These
7 results therefore confirm that storage organs can contribute to short-term plant re-growth
8 (Klimešová and Klimeš, 2007; Carter *et al.*, 2012). However, at a longer time scale, *P.*
9 *pratensis* recovered better than *T. flavescens* and was able to extend its root system (RRI > 1,
10 Fig. 6C). Higher ion solutes, WSC contents in roots, and root development after drought
11 treatment (Jiang and Huang, 2000) may explain the better recovery capacity of *P. pratensis*
12 than of *T. flavescens* and *P. trivialis* (Fig. 6C).

13 All the species showed dehydration avoidance and tolerance mechanisms involving
14 sucrose accumulation and/or fructan maintenance in plant organs. Also, differences between
15 carbohydrate dynamics in response to drought were as high among the grasses as between the
16 grasses and the forb (*T. officinale*) (Fig. 3, Fig. S1, Fig. S2). The Mediterranean cultivar of *D.*
17 *glomerata* survived better than the ecotype of cocksfoot of upland origin, probably because of
18 the higher initial fructan level in its leaf meristems, confirming previous results for this
19 cultivar (Volaire, 1995; Volaire and Lelièvre, 2001). However, the recovery of *D. glomerata*
20 and its Mediterranean cultivar one year after drought was similar, emphasizing the capacity of
21 resilience of the native species after a severe drought.

22 This study on forage grassland species showed diversity in plant strategies to survive and
23 recover after severe drought, as observed by Craine *et al.* (2012) or Pérez Ramos *et al.* (2013).
24 Against our third hypothesis, no trade-off was found between high resource acquisition under
25 non-limiting conditions and drought survival within the plant material tested originating from

1 upland areas subjected to infrequent severe drought. This performance results mainly from
2 dehydration avoidance and tolerance strategies associated with a strong allocation of carbon
3 to the root system ensured by an efficient carbon acquisition at the whole plant level. It is also
4 noteworthy that plant survival observed two weeks after rehydration was not a good indicator
5 of one-year recovery. This emphasizes the need to assess recovery after drought at a longer
6 time scale and to take into consideration storage organs as key organs for resilience capacity
7 of species. Our results also suggest that most of the native forage species studied are able to
8 survive and recover from extreme drought but with various time lags. This wide range of
9 drought resistance strategies in native forage upland species raises the question of high
10 resilience of diverse plant communities for maintenance of grassland services after extreme
11 events.

12

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24

25

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APPENDIX

A1: List of abbreviations

Abbreviation	Variable measured	Units
CMS	Cell membrane stability	%
D ₉₅	95% rooting depth	cm
D _{max}	Maximum rooting depth	cm
HDP	fructans content with degrees of polymerization over 5 (high degree of polymerization)	mg g ⁻¹ DW
LDMC	Leaf dry matter content	g g ⁻¹
LDP	fructans content with degrees of polymerization 3 and 4 (low degree of polymerization)	mg g ⁻¹ DW
LER	Leaf elongation rate	mm d ⁻¹
LRWC	Leaf relative water content	%
LS	Leaf senescence	%
MWC	Leaf meristem water content	%
PET	Potential evapotranspiration	mm d ⁻¹
QWSCr	amount of WSCs in roots	mg per plant
RDMC	Root dry matter content	g g ⁻¹
RECOV	Plant one-year recovery	%
RI	Resilience index of spring forage production	-
RM	total root mass	kg m ⁻³
RRI	Resilience index of spring standing root mass	-
SD	Sampling date	-
SLA	Specific leaf area	m ² kg ⁻¹
SO	Sucrose content	mg g ⁻¹ DW
SURV	Plant survival rate	%
SWC	Soil water content	m ³ m ⁻³
SWC _{el}	Soil water content at the end of leaf elongation	m ³ m ⁻³
SWC _s	Soil water content for 50% leaf senescence	m ³ m ⁻³
WSC	Water soluble carbohydrate	mg g ⁻¹ DW
WU	Plant water used	g kg ⁻¹

Table 1. Plant traits measured under irrigation before drought (DOY 78-181), at the end of moderate (DOY 182-201) and severe (DOY 202-231) drought. Mean values are shown (n = 3). Letters show significant differences between the seven species, except for tap root of *To*, according to one-way ANOVA and Tukey tests (P < 0.05).

Periods	Traits	To _{taproot}	To	Md	Dg	Tf	Pp	Fa	Pt
Before drought	LDMC		168 c	259 ab	242 ab	91 d	311 a	223 bc	267 ab
	SLA		29.2 bc	24.9 bc	27.3 bc	33.5 b	22.8 c	24.5 c	49.5 a
	LER		9.4 abc	10.3 ab	11.8 a	6.7 c	4.6 c	6.6 bc	3.3 c
	LRWC ₁		96 ab	98 a	97 ab	96 ab	95 ab	96 ab	88 b
	MWC ₁		80 a	77 ab	80 a	75 ab	74 ab	80 a	69 b
	RM ₁	7.5	1.7 c	4.9 a	4.6 ab	3.3 b	3.6 bc	5.0 a	2.6 c
	RDMC ₁	0.257	0.101 d	0.211 ab	0.189 c	0.195 bc	0.228 a	0.206 bc	0.204 bc
	Dmax ₁		146 a	134 a	122 ab	70 cd	100 bc	146 a	57 d
	D95 ₁	47	98 a	82 ab	71 bc	62 bc	60 bc	106 a	48 c
	WU ₁		125 a	106 ab	111 ab	78 b	75 b	133 a	82 b
Moderate drought	LRWC ₂		69 a	53 ab	39 ab	30 b	29 b	33 b	27 b
	MWC ₂		63.0 a	47.8 bc	46.4 bc	40.5 cd	38.0 d	49.6 b	38.3 d
	LS ₂		20.8 a	6.7 a	19.2 a	20.8 a	12.5 a	23.3 a	54.2 b
	RM ₂	11.5	2.1 d	5.9 a	4.7 ab	3.7 cd	4.0 bc	4.9 ab	3.0 cd
	RDMC ₂	0.340	0.154 c	0.305 a	0.278 ab	0.243 b	0.286 ab	0.306 a	0.302 ab
	Dmax ₂		145 a	129 ab	134 a	78 c	95 bc	147 a	67 c
	D95 ₂	53	91 a	74 bc	67 c	58 c	67 c	98 ab	49 c
	WU ₂		103	75	76	46	47	70	37
SWC _{el}		0.122	0.100	0.110	0.071	0.065	0.099	0.062	
Severe drought	LRWC ₃		16	28	19	11	16	-	-
	MWC ₃		43.8 a	46.2 a	35.5 ab	28.5 ab	26.3 ab	22.2 ab	14.6 b
	LS ₃		90 bc	81 c	96 ab	97 ab	90 bc	100 a	100 a
	RM ₃	13.0	1.8 c	5.3 a	4.9 a	3.6 b	3.7 b	5.0 a	2.8 bc
	RDMC ₃	0.431	0.237 b	0.343 a	0.339 a	0.266 ab	0.291 ab	0.326 a	0.371 a
	Dmax ₃		149 a	133 a	136 a	85 b	91 b	150 a	61 b
	D95 ₃	57	113 a	78 bc	67 cd	61 cd	53 d	93 ab	56 cd
	WU ₃		70	28	36	20	40	20	19
SWC _s		0.085	0.052	0.092	0.029	0.018	0.053	0.061	

Dmax: maximum rooting depth (cm); D95: 95% rooting depth (cm); LER: leaf elongation rate (mm d⁻¹); LDMC: leaf dry matter content (g g⁻¹); LRWC: leaf relative water content (%); LS: leaf senescence (%); MWC: leaf meristem water content (%); RM: total root mass (kg m⁻³); RDMC: root dry matter content (g g⁻¹); SWC_{el}: soil water content at the end of leaf growth (m³ m⁻³); SLA: specific leaf area (m² kg⁻¹); SWC_s: soil water content at 50% of leaf senescence (m³ m⁻³); WU: water used (g kg⁻¹). Lower script (1, 2, 3) of traits when present corresponds to sampling dates (SD). Dg: *Dactylis glomerata*; Fa: *Festuca arundinacea*; Md: *Dactylis glomerata* 'Medly'; Pp: *Poa pratensis*; Pt: *Poa trivialis*; Tf: *Trisetum flavescens*; To: *Taraxacum officinale*.

1 **Table 2.** Effects of moderate drought (SD1 vs. SD2) and severe drought (SD2 vs. SD3) on
 2 plant traits. Degrees of freedom (d.f.) and F-values are shown. Symbols indicate significant
 3 effect after one-way ANOVA: SD: sampling date; Sp: species; ⁺: P ≤ 0.1; * P ≤ 0.05; **: P ≤
 4 0.01; ***: P ≤ 0.001; ns: non-significant.

	Moderate drought				Severe drought			
	d.f.	SD	Sp	SD x Sp	d.f.	SD	Sp	SD x Sp
LRWC	22	25.2***	6.1***	7.8***	17	26.1***	2.1	1.3
MWC	27	811.1***	19.9***	4.4**	26	47.4***	8.4***	2.1+
LS	49	198.7***	11.7***	0.8	70	660.2***	16.0***	2.4*
RM	32	5.6*	32.1***	0.7	28	1.0	30.1***	0.4
RDMC	28	242.1***	51.3***	2.9*	28	46.6***	21.2***	0.6
Dmax	28	0.6	47.4***	0.5	28	0.13	44.2***	0.2
D95	28	0.6	9.9***	0.3	28	0.3	10.1***	0.9
WU	21	62.4***	13.4***	1.3	14	49.0***	10.4***	1.7
WSCm	26	0.1	2.7*	0.5	26	0.1	2.1+	1.0
HDPm	26	1.4	4.7**	1.3	26	0.4	5.3**	1.7
LDPm	26	5.7*	6.1***	1.6	26	0.4	4.7**	0.2
SOM	26	27.9***	2.3 ⁺	1.5	26	0.13	3.5*	0.8
GOM	26	8.0**	6.0***	1.0	26	48.4***	1.8	2.5+
FOM	26	2.3	10.3***	1.4	26	0.2	22.5***	1.5
WSCr	27	1.7	37.7***	6.2***	28	120.5***	41.2***	3.6**
HDPr	27	8.3**	51.7***	6.1***	28	96.6***	56.4***	3.7**
LDPr	27	30.0***	22.3***	2.1 ⁺	28	47.3***	18.1***	0.9
SOR	27	11.0**	5.2**	4.2**	28	75.1***	10.9***	0.5
GOR	27	67.1***	13.3***	1.2	28	98.3***	17.6***	2.4+
FOR	27	0.2	68.1***	0.8	28	4.0+	19.1***	1.1
WSCa	22	0.8	7.9***	3.5*	24	6.1*	10.8***	2.2+
HDPa	22	0.1	10.6***	4.2**	24	2.3	14.3***	2.4+
LDPa	22	0.5	8.9***	2.4 ⁺	24	4.8*	11.8***	3.2*
SOa	22	9.3**	3.8**	2.8*	24	14.8***	6.5***	2.3+
GOa	22	8.2**	7.9***	4.3**	24	32.4***	13.2***	2.5+
FOa	22	0.3	4.9**	0.8	24	9.1**	11.3***	1.4
WSCo	11	2.3	35.4***	0.6	14	10.3**	24.4***	5.0*
HDPo	11	4.0 ⁺	34.7***	0.4	14	13.1**	26.4***	4.2*
LDPo	11	4.8+	562.8***	0.2	14	0.2	121.3***	0.8
SOo	11	21.2***	8.9**	4.3*	16	4.7*	15.0***	4.0*
GOo	11	35.4***	7.9**	1.8	14	43.1***	18.9***	3.0+
FOo	11	0.1	18.1***	4.0*	14	0.1	18.1***	4.0*

5 Dmax: maximum rooting depth (cm); D95: 95% rooting depth (cm); FO: fructose content in leaf meristems
 6 (m), roots (r), root apices (a) and storage organs (o) (mg g⁻¹ DM); GO: glucose content in leaf meristems
 7 (m), roots (r), root apices (a) and storage organs (o) (mg g⁻¹ DM); HDP: fructan DP ≥ 5 content in leaf
 8 meristems (m), roots (r), root apices (a) and storage organs (o) (mg g⁻¹ DM); LDP: fructans DP3-4 content
 9 in leaf meristems (m), roots (r), root apices (a) and storage organs (o) (mg g⁻¹ DM); LER: leaf elongation
 10 rate (mm d⁻¹); LRWC: leaf relative water content (%); LS: leaf senescence (%); MWC: water content of
 11 leaf meristems (%); RDMC: root dry matter content (g g⁻¹); RM: total root mass (kg m⁻³); SO: sucrose
 12 content in leaf meristems (m), roots (r), root apices (a) and storage organs (o) (mg g⁻¹ DM); WSC: water
 13 soluble carbohydrate content in leaf meristems (m), roots (r), root apices (a) and storage organs (o) (mg g⁻¹
 14 DM); WU: water used (g kg⁻¹).

17 **Table 3.** Pearson's correlation coefficients between all pairs of plant traits measured under
 18 irrigation (DOY 78–181), moderate drought (DOY 182–201) and severe drought (DOY 202–
 19 231) treatments. Significant correlations are shown in bold ($P < 0.05$) and underlined
 20 characters ($P < 0.001$).

	SURV	RECOV	RI	RRI
LER	<u>0.68</u>	0.35	0.26	0.32
SLA	-0.38	-0.38	<u>-0.76</u>	-0.38
RM ₁	<u>0.62</u>	0.31	0.05	-0.14
Dmax ₁	0.53	0.41	0.48	0.18
D95 ₁	0.00	0.01	0.47	0.01
RDMC ₁	-0.42	-0.10	0.48	0.08
LDMC	-0.19	0.29	0.35	0.26
WU ₁	0.32	0.23	0.29	0.05
LRWC ₂	<u>0.67</u>	0.39	0.10	0.31
RDMC ₂	-0.51	-0.27	0.34	0.14
RM ₂	<u>0.65</u>	0.35	-0.08	-0.02
WU ₂	<u>0.72</u>	0.43	0.23	0.20
HDPm ₂	0.28	0.07	0.21	0.10
SOM ₂	-0.50	-0.22	0.23	-0.14
HDP _{r2}	<u>0.60</u>	0.26	0.10	0.02
SOR ₂	0.26	0.06	0.32	0.15
HDPa ₂	0.09	-0.02	-0.13	-0.05
SOa ₂	0.30	0.22	0.03	0.01
SWCel	0.45	0.46	0.14	0.49
LS3	<u>-0.70</u>	-0.24	-0.32	-0.25
SWCs	-0.42	-0.22	<u>-0.72</u>	-0.25
MWC3	<u>0.76</u>	0.34	0.31	0.21
CMSm	0.51	0.09	0.37	0.09
CMSr	0.06	-0.09	0.50	0.09
CMSa	0.36	0.17	0.39	0.38
HDPm3	0.07	0.39	0.40	0.42
SOM3	-0.35	0.08	0.05	0.21
HDP _{r3}	0.29	0.13	0.32	0.20
SOR3	0.56	0.25	0.25	0.08
HDPa3	0.04	-0.05	0.47	0.21
SOa3	-0.10	-0.11	<u>0.65</u>	0.04
QWSCr	0.30	0.14	<u>0.61</u>	0.36
SURV	1	0.28	0.12	0.24
RECOV		1	0.40	<u>0.66</u>
RI			1	<u>0.55</u>
RRI				1

21 Dmax: maximum rooting depth (cm); D95: 95% rooting depth (cm); HDP: fructan DP ≥ 5 content in leaf
 22 meristems (m), roots (r) and root apices (a) (mg g^{-1} DM); LER: leaf elongation rate (mm d^{-1}); LDMC: leaf dry
 23 matter content (g g^{-1}); LRWC: leaf relative water content (%); LS: leaf senescence (%); MWC: leaf meristem
 24 water content (%); QWSCr: amount of WSC in roots (mg); RI: resilience index of spring forage production;
 25 RECOV: one-year recovery of aerial green tissue (%); RRI: resilience index of standing root mass; RM: total
 26 root mass (kg m^{-3}); RDMC: root dry matter content (g g^{-1}); SO: sucrose content in leaf meristems (m), roots (r),
 27 and root apices (a) (mg g^{-1} DM); SURV: plant survival rate two weeks after rehydration (%); SWCel: soil water
 28 content at the end of leaf growth ($\text{m}^3 \text{m}^{-3}$); SLA: specific leaf area ($\text{m}^2 \text{kg}^{-1}$); SWCs: soil water content at 50% of
 29 leaf senescence ($\text{m}^3 \text{m}^{-3}$); WU: water used (g kg^{-1}). Lower script (1, 2, 3) of traits when present corresponds to
 30 sampling dates (SD).
 31
 32
 33

34 **Figure legends**

35 **Figure 1.** Time course of soil volumetric water content (SWC, $\text{m}^3 \text{m}^{-3}$) for *Taraxacum*
36 *officinale* (A), *Dactylis glomerata* ‘Medly’ (B), *Dactylis glomerata* (C), *Trisetum flavescens*
37 (D), *Poa pratensis* (E), *Festuca arundinacea* (F), *Poa trivialis* (G), irrigated and during
38 drought treatment, at 10 cm (solid lines), 40 cm (dashed lines) and 100 cm (dotted lines)
39 below soil surface. Vertical lines represent last watering (black solid line), end of leaf growth
40 (grey solid line) and rehydration (black dashed line), delimiting periods of moderate drought
41 (DOY 182-201) and severe drought (DOY 202-231), respectively. Arrows mark the four
42 sampling dates: SD1 (DOY 180); SD2 (DOY 200); SD3 (DOY 235); SD4 (DOY 242).

43
44 **Figure 2.** Root mass (kg m^{-3}) profile in the soil of *Taraxacum officinale* (A), *Dactylis*
45 *glomerata* ‘Medly’ (B), *Dactylis glomerata* (C), *Trisetum flavescens* (D), *Poa pratensis* (E),
46 *Festuca arundinacea* (F), *Poa trivialis* (G) measured before drought treatment (black circles),
47 during moderate drought (white circles) and severe drought (white squares), and in the control
48 plants at the end of drought (black squares). For *Taraxacum officinale*, insert corresponds to
49 tap root mass profile. Mean values are shown \pm se (n = 3).

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51 **Figure 3.** Fructans with degree of polymerization ≥ 5 (HDP) (A, C, E, G) and sucrose
52 contents (B, D, F, H) in leaf meristems (A, B), in roots (C, D), in root apices (E, F) of
53 *Taraxacum officinale* (filled orange triangles), *Dactylis glomerata* ‘Medly’ (open blue
54 circles), *Dactylis glomerata* (filled blue circles), *Trisetum flavescens* (open red triangles), *Poa*
55 *pratensis* (filled green squares), *Festuca arundinacea* (filled red triangles), *Poa trivialis* (open
56 green squares) and in storage organs (G, H) of *Taraxacum officinale* (taproot), *Trisetum*
57 *flavescens* (stolon), *Poa pratensis* (rhizome) and *Poa trivialis* (stolon) during the experimental
58 drought (DOY 182 – 231) and in the control plants (DOY 242). Mean values are shown \pm se
59 (n = 3). Symbols indicate significant differences between species after one-way ANOVA: $^+ P$
60 ≤ 0.1 ; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

61
62 **Figure 4.** Cell membrane stability measured in leaf meristems (A), roots (R), and root apices
63 (C) of *Taraxacum officinale* (To), *Dactylis glomerata* ‘Medly’ (Md), *Dactylis glomerata*
64 (Dg), *Trisetum flavescens* (Tf), *Poa pratensis* (Pp), *Festuca arundinacea* (Fa), *Poa trivialis*
65 (Pt), and in storage organs (D) of To (taproot), Tf (stolon), Pp (rhizome) and Pt (stolon)
66 measured at the end of severe drought in control (black) and drought (white) plants. Mean

67 values \pm se are shown (n = 3). Symbols indicate significant effect of drought treatment on
68 plant organs for each species: *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$.

69

70 **Figure 5.** Correlations between cell membrane stability and fructans with degree of
71 polymerization ≥ 5 (high degree of polymerization, HDP) (A, C, E), and sucrose contents (B,
72 D, F) measured in leaf meristems (A, B), in roots (C, D) of *Taraxacum officinale* (filled
73 orange triangles), *Dactylis glomerata* ‘Medly’ (open blue circles), *Dactylis glomerata* (filled
74 blue circles), *Trisetum flavescens* (open red triangles), *Poa pratensis* (filled green squares),
75 *Festuca arundinacea* (filled red triangles), *Poa trivialis* (open green squares) and in storage
76 organs (e, f) of *Taraxacum officinale* (taproot), *Trisetum flavescens* (stolon), *Poa pratensis*
77 (rhizome) and *Poa trivialis* (stolon) measured at the end of the severe drought in control and
78 drought plants. Lines represent the significant linear regressions ($P < 0.05$) for all species
79 (black line) and for each species (To: orange line; Md: dashed blue line; Dg: solid blue line;
80 Tf: dashed red line; Pp: solid green line; Fa: solid red line; Pt: dashed green line).

81

82 **Figure 6.** Pictures of *Taraxacum officinale* (To), *Dactylis glomerata* ‘Medly’ (Md), *Dactylis*
83 *glomerata* (Dg), *Trisetum flavescens* (Tf), *Poa pratensis* (Pp), *Festuca arundinacea* (Fa), *Poa*
84 *trivialis* (Pt) two weeks after rehydration (A) and one year after rehydration on drought plants
85 (B), canopy greenness of the tissue (%) two weeks after rehydration (white bars) and one year
86 (hashed bars) after rehydration (C), and resilience index of spring forage production (D) and
87 standing root mass (D) of the seven species. Mean values \pm se are shown (n = 3). Symbols
88 indicate significant effect of periods on canopy green tissue, spring forage production and
89 standing root mass for each species (+: $P < 0.1$; *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$).
90 Different letters correspond to between-species differences for canopy green tissue measured
91 two weeks after rehydration.

92

93 **Figure 7.** Analyses of principal component combining traits related to strategies of resource
94 acquisition (A), dehydration avoidance (B), and dehydration tolerance (C), measured on
95 *Taraxacum officinale* (To), *Dactylis glomerata* ‘Medly’ (Md), *Dactylis glomerata* (Dg),
96 *Trisetum flavescens* (Tf), *Poa pratensis* (Pp), *Festuca arundinacea* (Fa), *Poa trivialis* (Pt)
97 under full irrigation (SD1), during moderate drought (SD2) and severe drought (SD3), and
98 after rehydration. CMSa, CMSm, CMSr: cell membrane stability in root apices, leaf
99 meristems and roots; Dmax: maximum rooting depth; HDPm, HDPs: fructans content with
100 degree of polymerization ≥ 5 in leaf meristems and roots; LER: leaf elongation rate; LDMC:

101 leaf dry matter content; LRWC: leaf relative water content; LS: leaf senescence; MWC: leaf
102 meristem water content; RDMC: root dry matter content; RECOV: one-year recovery of
103 aerial green tissue; RI: resilience index of spring forage production; RM: root mass; RRI:
104 resilience index of standing root mass; SLA: specific leaf area; S_{Om}, S_{Or}: sucrose content in
105 leaf meristems and roots; SURV: plant survival two weeks after rehydration; WU: water used.
106 Lower script (1, 2, 3) of traits when present corresponds to sampling dates (SD).

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135 **Supplementary data**

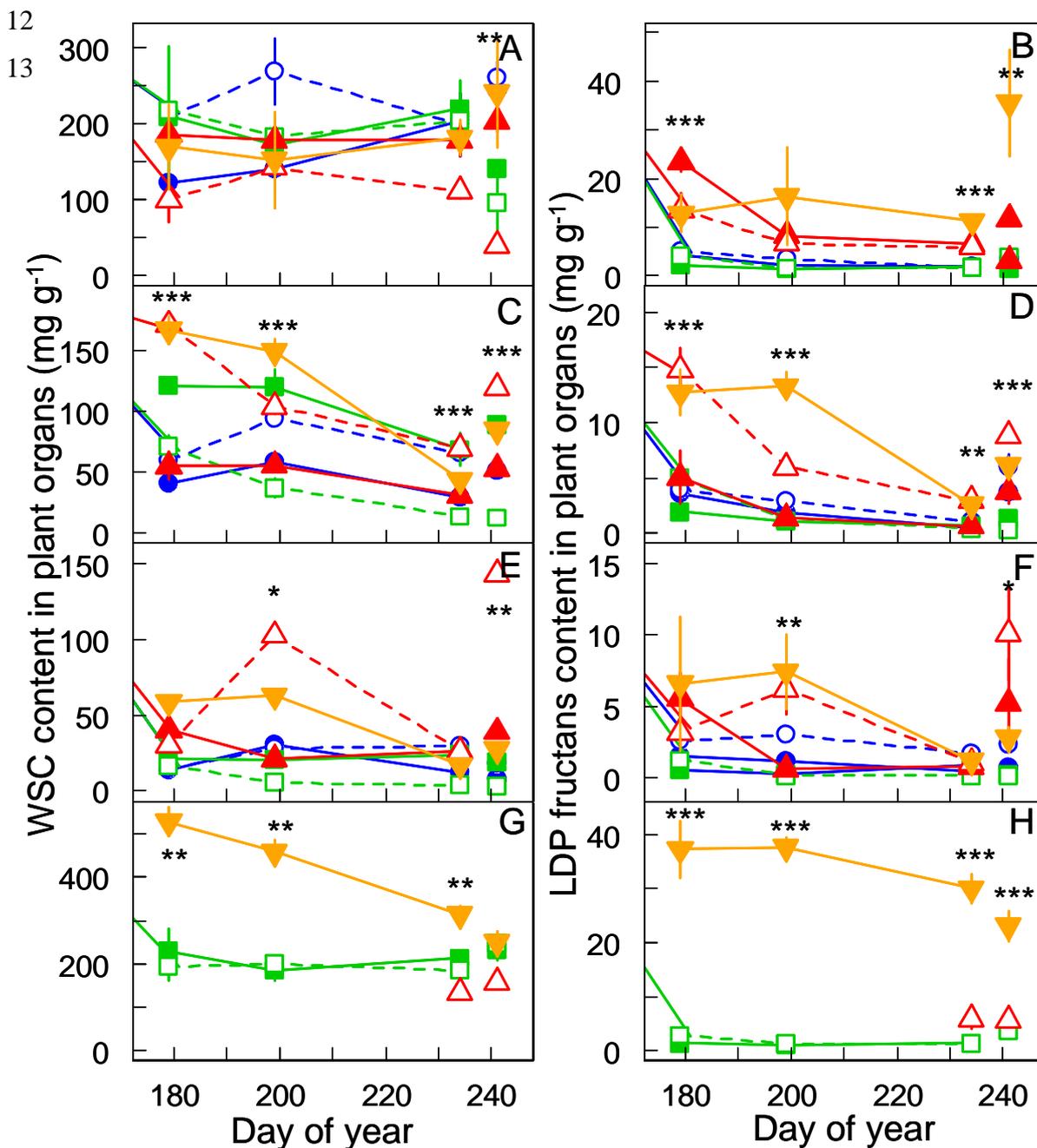
136 **Table S1.** Climatic conditions during the experiment: before drought in spring (DOY 78–
 137 181), moderate drought (DOY 182–201) and severe drought (DOY 202–231), during the first
 138 two weeks of rehydration (DOY 232–246) and the recovery period in 2011 (DOY 247–365)
 139 and 2012 (DOY 1-158).

Year	Period	Mean	Minimum	Maximum	Global		
		T _{air} (°C)	T _{air} (°C)	T _{air} (°C)	radiation (J cm ⁻²)	PET (mm d ⁻¹)	PET (mm period ⁻¹)
2011	Before drought	15.2	9.2	21.6	1967	3.8	393
	Moderate drought	18.3	11.8	23.9	1980	4.1	83
	Severe drought	19.4	13.9	25.3	1853	4	121
	Rehydration	20.9	15	27.8	1753	3.9	58
	Recovery	12	7.4	17.3	860	1.4	161
2012	Recovery	8.1	3.3	13.1	1203	2.0	323

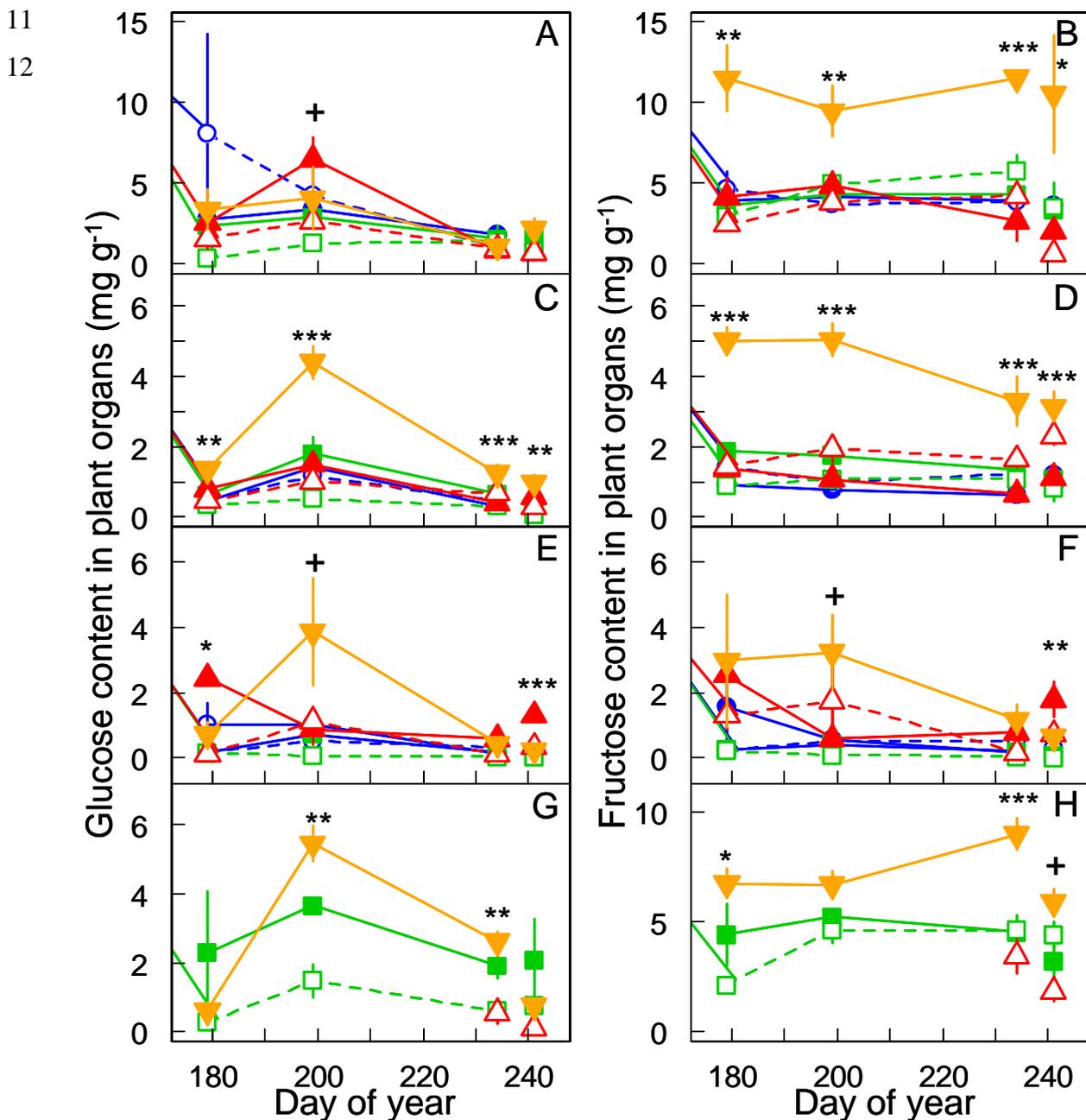
140 PET: Potential evapotranspiration.

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1 **Figure S1.** Total water-soluble carbohydrate (WSC) (A, C, E, G) and fructans with degrees of
 2 polymerization 3 and 4 (low degree of polymerization, LDP) contents (B, D, F, H) in leaf
 3 meristems (A, B), roots (C, D), root apices (E, F) of *Taraxacum officinale* (filled orange
 4 triangles), *Dactylis glomerata* ‘Medly’ (open blue circles), *Dactylis glomerata* (filled blue
 5 circles), *Trisetum flavescens* (open red triangles), *Poa pratensis* (filled green squares),
 6 *Festuca arundinacea* (filled red triangles), *Poa trivialis* (open green squares) and in storage
 7 organs (G, H) of *Taraxacum officinale* (taproot), *Trisetum flavescens* (stolon), *Poa pratensis*
 8 (rhizome) and *Poa trivialis* (stolon) during the experimental drought (DOY 182 – 231) and in
 9 the control plants (DOY 242). Mean values are shown \pm se (n = 3). Symbols indicate
 10 significant differences between species after one-way ANOVA: $^+$ $P \leq 0.1$; * $P \leq 0.05$; **: $P \leq$
 11 0.01; ***: $P \leq 0.001$.



1 Figure S2. Glucose (A, C, E, G) and fructose contents (B, D, F, H) in leaf meristems (A, B),
 2 roots (C, D), root apices (E, F) of *Taraxacum officinale* (filled orange triangles), *Dactylis*
 3 *glomerata* ‘Medly’ (open blue circles), *Dactylis glomerata* (filled blue circles), *Trisetum*
 4 *flavescens* (open red triangles), *Poa pratensis* (filled green squares), *Festuca arundinacea*
 5 (filled red triangles), *Poa trivialis* (open green squares) and in storage organs (G, H) of
 6 *Taraxacum officinale* (taproot), *Trisetum flavescens* (stolon), *Poa pratensis* (rhizome) and
 7 *Poa trivialis* (stolon) during the experimental drought (DOY 182-231) and in the control
 8 plants (DOY 242). Mean values are shown \pm se (n = 3). Symbols indicate significant effect of
 9 drought treatment on plant organs for each species: +: $P \leq 0.1$, *: $P \leq 0.05$, **: $P \leq 0.01$, ***:
 10 $P \leq 0.001$.



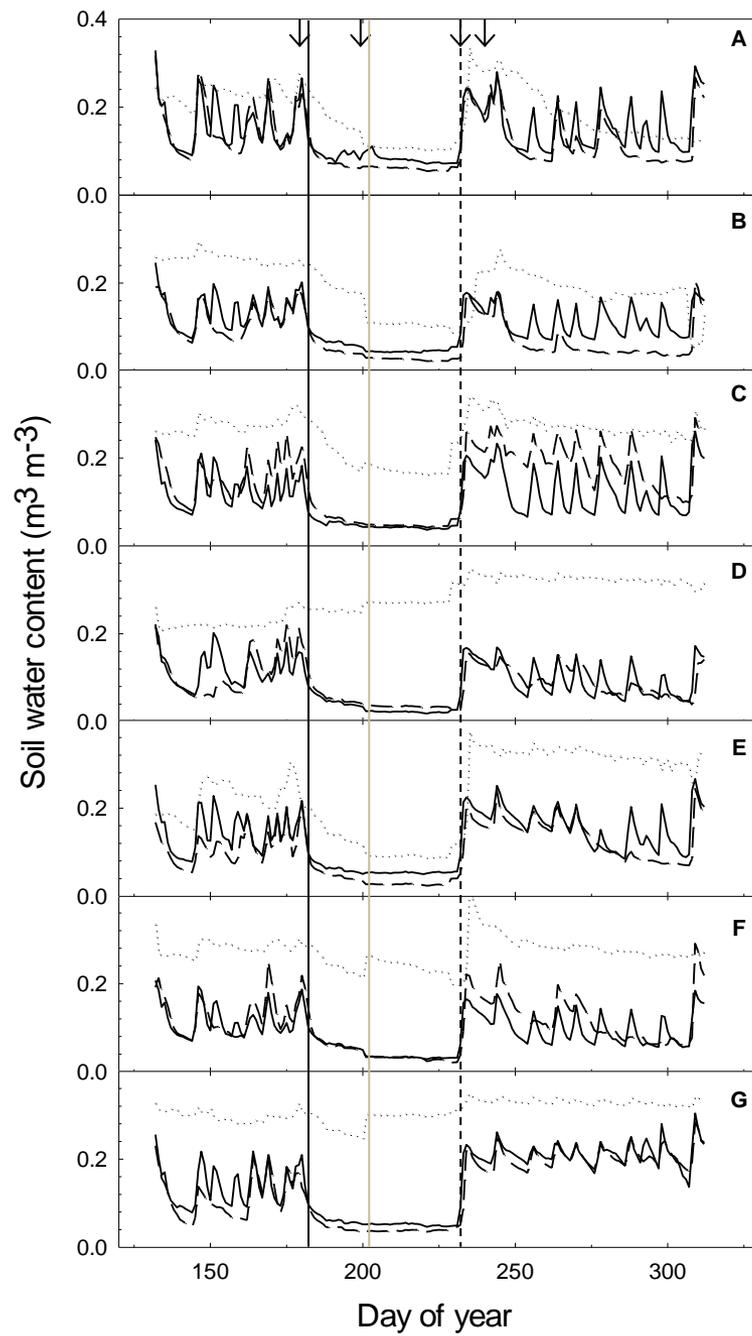


Figure 2

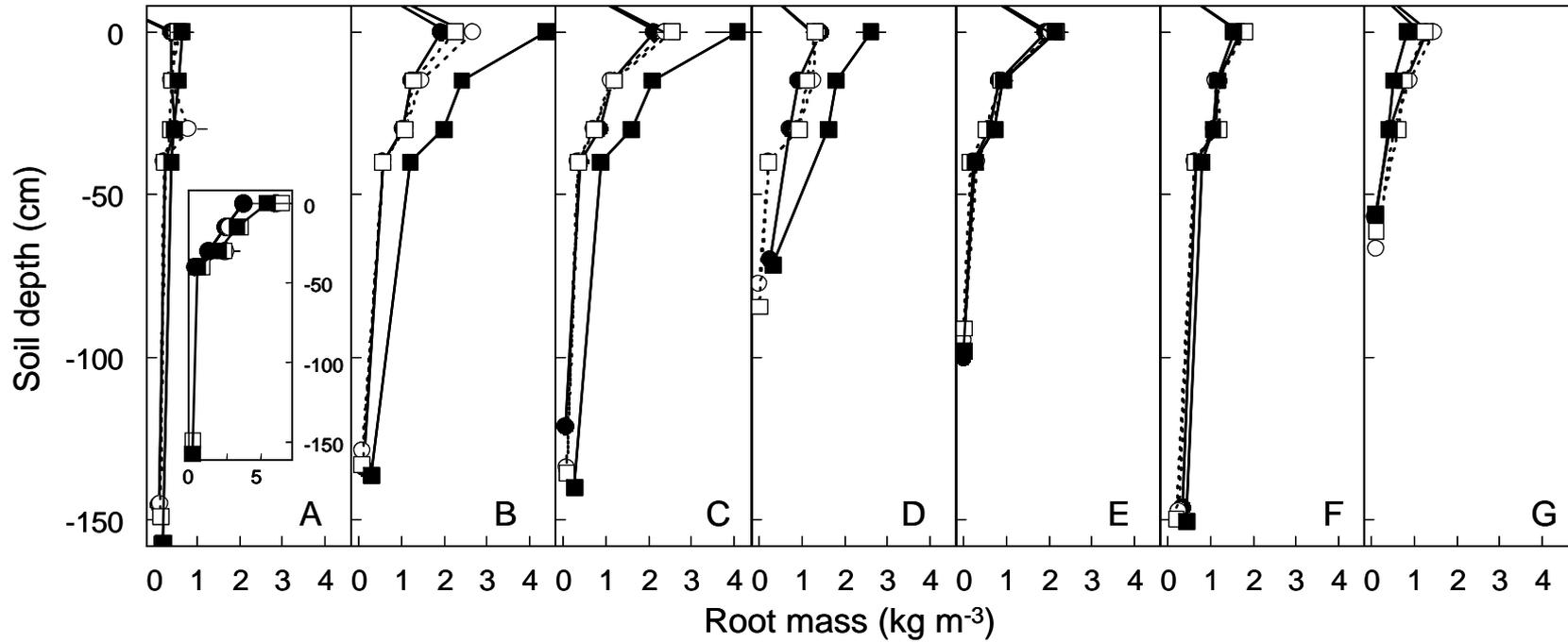


Figure 3

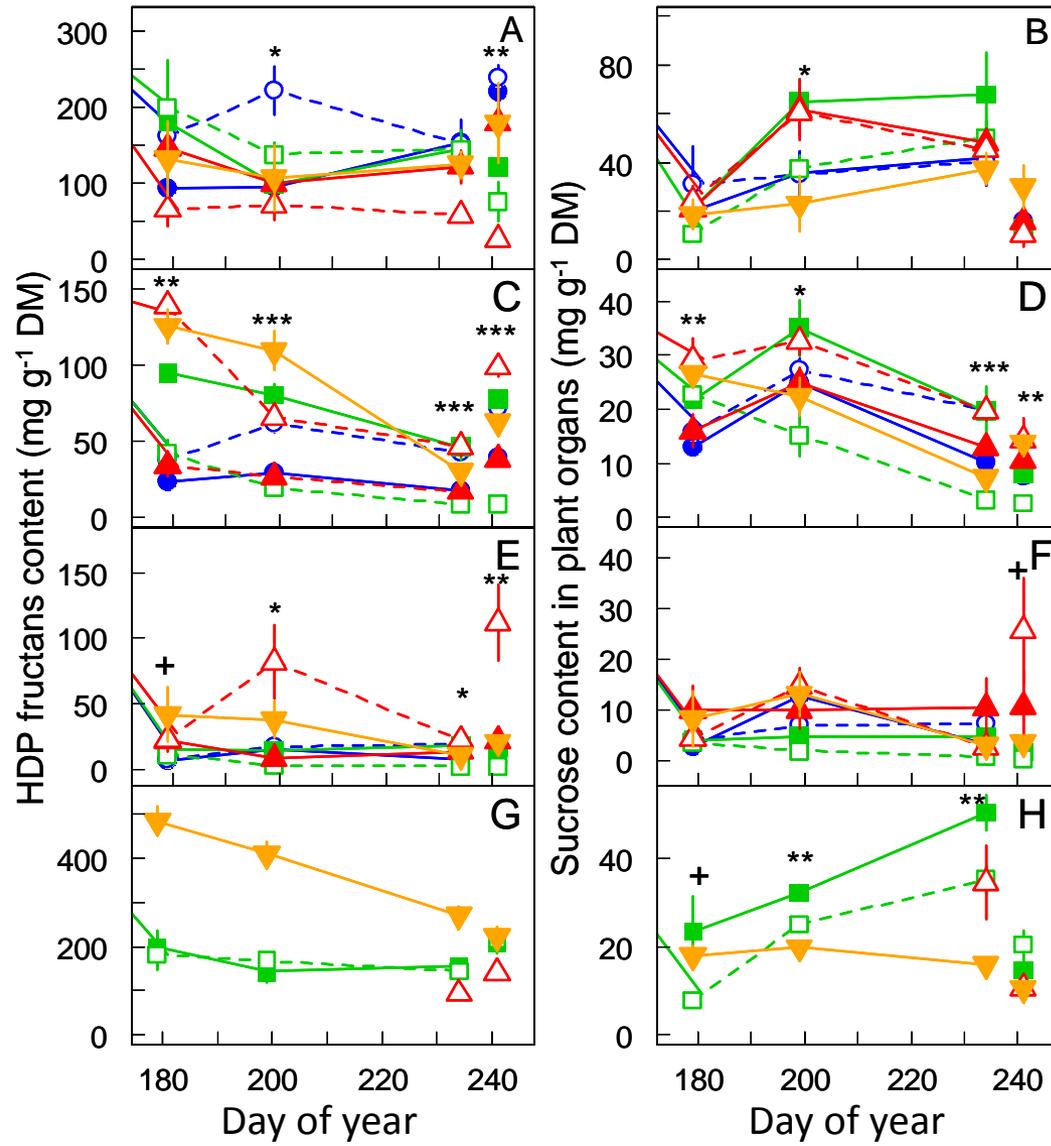


Figure 4

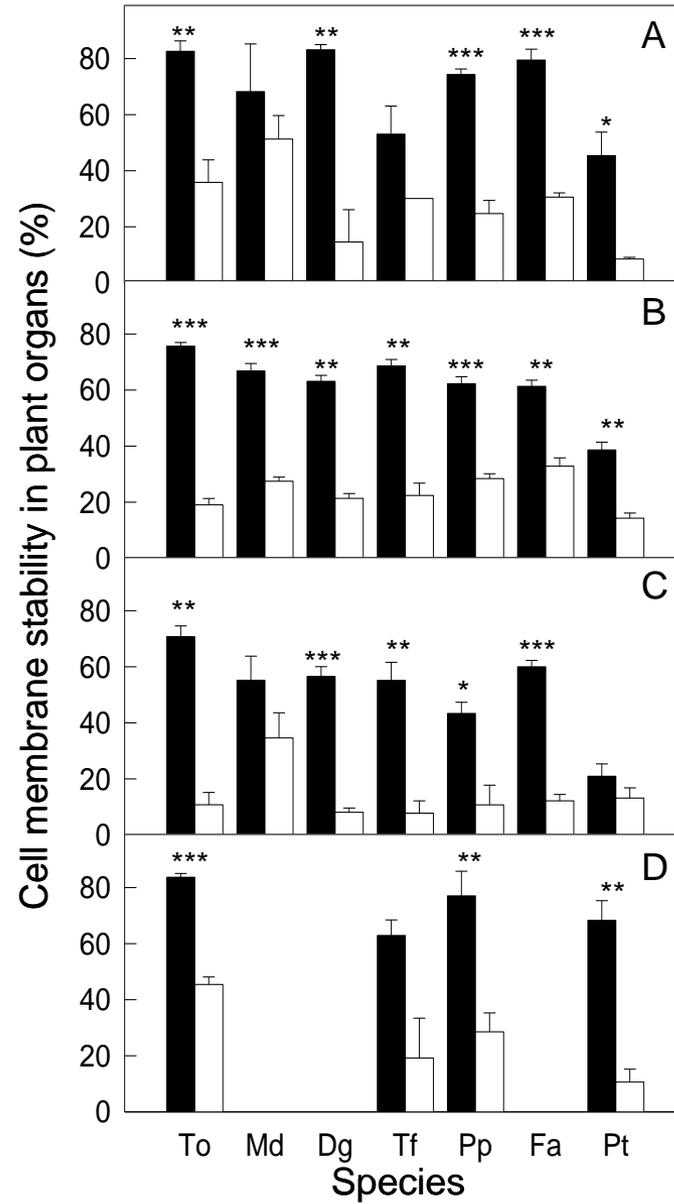


Figure 5

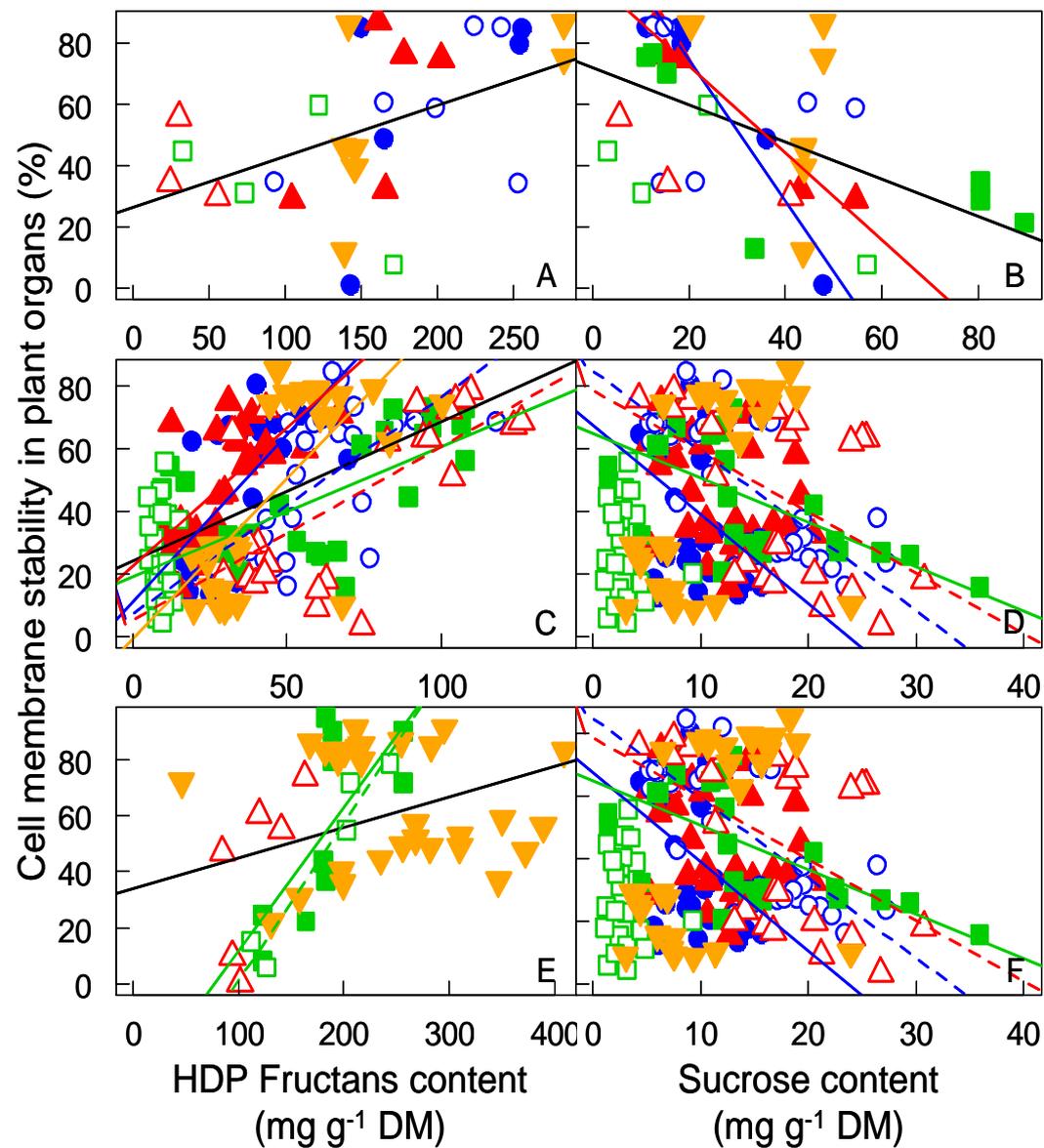


Figure 6

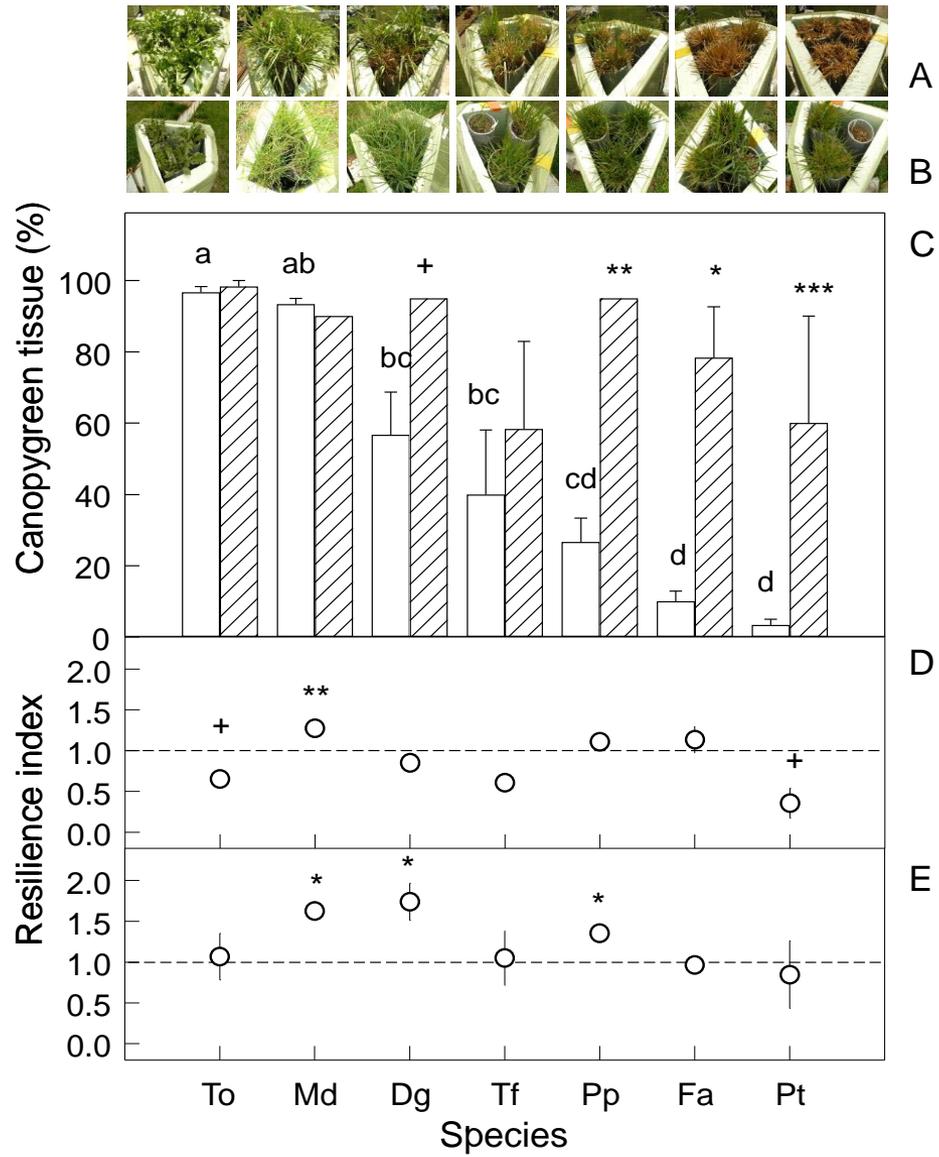


Figure 7

