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RESEARCH PAPER

The resilience of perennial grasses under two climate scenarios is correlated with carbohydrate metabolism in meristems

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Abstract

Extreme climatic events (ECEs) such as droughts and heat waves affect ecosystem functioning and species turnover. This study investigated the effect of elevated CO_2 on species' resilience to ECEs. Monoliths of intact soil and their plant communities from an upland grassland were exposed to 2050 climate scenarios with or without an ECE under ambient (390 ppm) or elevated (520 ppm) CO_2. Ecophysiological traits of two perennial grasses (*Dactylis glomerata* and *Holcus lanatus*) were measured before, during, and after ECE. At similar soil water content, leaf elongation was greater under elevated CO_2 for both species. The resilience of *D. glomerata* increased under enhanced CO_2 (+60%) whereas *H. lanatus* mostly died during ECE. *D. glomerata* accumulated 30% more fructans, which were more highly polymerized, and 4-fold less sucrose than *H. lanatus*. The fructan concentration in leaf meristems was significantly increased under elevated CO_2. Their relative abundance changed during the ECE, resulting in a more polymerized assemblage in *H. lanatus* and a more depolymerized assemblage in *D. glomerata*. The ratio of low degree of polymerization fructans to sucrose in leaf meristems was the best predictor of resilience across species. This study underlines the role of carbohydrate metabolism and the species-dependent effect of elevated CO_2 on the resilience of grasses to ECE.

Keywords: *Dactylis glomerata*, elevated CO_2, extreme climatic event, fructans, *Holcus lanatus*, resilience, sucrose.

Introduction

Grasslands are the most important agro-ecosystems worldwide; they deliver many ecosystem services, ranging from forage supply to soil carbon storage and biodiversity preservation (Pilgrim *et al.*, 2010; Gaujour *et al.*, 2012). However, climate change caused by increased greenhouse gas emissions in the atmosphere may jeopardize the stability and functions of many ecosystems (Intergovernmental Panel on Climate Change, 2014). Reduced precipitation coupled with increased temperatures will lead to more frequent and intense droughts (Tubiello *et al.*, 2007; Dai, 2011) and severe heat waves (Trnka *et al.*, 2011; Orlowsky and Seneviratne, 2012; Seneviratne *et al.*, 2014). The interactions between these combined climatic factors result in complex responses that challenge our current understanding and will affect plant physiological processes in a way that cannot be predicted from single-factor treatments (Albert *et al.*, 2011*a*; Dieleman *et al.*, 2012; Mueller *et al.*, 2016). An important issue is to explore to what extent extreme climatic events (ECEs, as defined by Smith, 2011*a*) will interact with the predicted rise in CO_2, in particular, regarding their impact on plant mortality and therefore on overall ecosystem resilience (Way, 2013; Niu *et al.*, 2014; Felton and Smith, 2017).

Many studies have explored the role of moderate water deficits on plant water status and carbon uptake under elevated CO_2 concentrations (eCO_2) (Clark *et al.*, 1999; Korner, 2000; Volk *et al.*, 2000; Knapp *et al.*, 2001; Morgan *et al.*, 2004; Grant *et al.*, 2014). Generally, eCO_2 enhanced plant biomass production and improved water relations under drought (Clark *et al.*, 1999; Ghannoum *et al.*, 2000; Robredo *et al.*, 2011). In addition, depending on the warming intensity (Dieleman *et al.*, 2012), eCO_2 alleviated the drought response in a range of grassland species (Naudts *et al.*, 2013), and mitigated the impacts of climate extremes on the growth and production of *Arabidopsis thaliana* (Zinta *et al.*, 2014). Conversely, the stimulatory effects of eCO_2 on growth and photosynthesis may decline due to increases in temperature stimulating photorespiration (Ziska and Bunce, 1994) or due to water and nitrogen limitations (Reich *et al.*, 2014). However, the effects of combined severe climatic extremes and CO_2 concentration on the ability of perennial herbaceous plants to survive and recover from severe stress have been explored relatively little (Roy *et al.*, 2016).

The accumulation of water-soluble carbohydrates (WSCs) under eCO_2 may contribute to increases in both drought resistance, that is, the maintenance of leaf growth under moderate water deficit, and also drought survival under severe water deficit (Roy *et al.*, 2016; Volaire, 2018). Under severe drought, once complete leaf senescence is reached, the dehydration tolerance of meristematic tissues of shoots and roots ensures plant survival by maintaining cell integrity through cell membrane stabilization (Volaire *et al.*, 2014; Zwicke *et al.*, 2015). WSCs, particularly fructans (soluble sucrose-derived fructose polymers), contribute to dehydration tolerance (Volaire, 1995; Livingston *et al.*, 2009) through cellular protection by membrane stabilization (Hincha *et al.*, 2007) and reactive oxygen species (ROS) scavenging (Peshev *et al.*, 2013). These WSC reserves also play a role during recovery, since they are hydrolysed to fuel regrowth after rehydration (Amiard *et al.*, 2004). Under eCO_2, temperate grassland species generally accumulated more WSCs, but a high variability of responses between species has been reported (Casella and Soussana, 1997; Allard *et al.*, 2003; Dumont *et al.*, 2015). Moreover, climate extremes under eCO_2 also elicited larger increases in the quantities of WSCs including fructans in grasses (AbdElgawad *et al.*, 2014). Since the protective properties of WSCs differ among compounds, in terms of both membrane stabilization (Hincha *et al.*, 2007) and ROS scavenging (Morelli *et al.*, 2003), the biochemical composition of the WSC pool should be considered. Indeed, temperature conditions can modify not only the amount of WSCs but also their composition (Abeynayake *et al.*, 2015), such as the distribution of fructan polymers [i.e. the relative content of low and high degree of polymerization (DP) fructans].

An experimental approach seems best suited to explore the impacts of combined environmental factors on plant responses (Reyer *et al.*, 2013). At the Montpellier (France) Ecotron, eCO_2 was shown to enhance both the resistance and the short-term overall resilience of an upland grassland community submitted to an ECE consisting of a severe edaphic drought and air warming (Roy *et al.*, 2016). Nevertheless, plant responses to eCO_2 are species-specific (Reich *et al.*, 2001; Roumet *et al.*, 2002; Teyssonneyre *et al.*, 2002b; Maestre *et al.*, 2007). Exploring the responses of dominant species to climate extremes will be key for predicting ‘winner’ and ‘loser’ species (Dukes, 2007) and therefore future ecosystem dynamics and function (Hoover *et al.*, 2014; De Boeck *et al.*, 2018). Temperature regimes interact with eCO_2 to affect plant physiology and growth according to plant functional groups; for instance, C_3 plants are more responsive than C_4 species (Wang *et al.*, 2012). Water stress and eCO_2 also interact to affect in different ways the growth of grasses that have contrasting drought strategies (Fernandez *et al.*, 2002; Wullschleger *et al.*, 2002). The botanical composition of temperate grasslands was shown to be modified under eCO_2 (Teyssonneyre *et al.*, 2002*b*), and changes in species abundance could result from differential mortality of different species under ECE (Grant *et al.*, 2014). Overall, in combination with an ECE, eCO_2 may differentially affect species or plant functional groups (Morgan *et al.*, 2011) and/or have a mitigating effect, particularly on drought survival (Volaire, 2018) and growth recovery after drought, as was shown at the community level (Roy *et al.*, 2016).

This study was designed within an experiment conducted at the ecosystem level (Roy *et al.*, 2016) to analyse the effect of eCO_2 on the resistance and resilience to an ECE of two coexisting C_3 grass species (*Dactylis glomerata* and *Holcus lanatus*). We investigated traits associated with nitrogen, carbon, and water use, at shoot level (biomass), leaf level (water potential, growth, nitrogen content), and surviving leaf meristem level (water status, membrane stability, non-structural carbohydrate metabolism). The chosen species are known to respond positively to eCO_2, but with higher drought resistance and survival for *Dactylis* (Teyssonneyre *et al.*, 2002*a, b*). The extent of the intraspecific variability in response to an ECE and eCO_2 was therefore tested according to the following hypotheses: (i) eCO_2 increases resistance to ECE (leaf growth maintenance during ECE) through higher water and WSC content; (ii) eCO_2 improves survival and biomass recovery and therefore resilience after ECE; and (iii) WSC metabolism and fructan composition in leaf meristems play a central role to support cell protection in the most severe stages of ECE, and contribute to plant resilience.

Materials and methods

Experimental design

The experimental design was previously described by Roy *et al.* (2016). The study tested the species’ response to future climate scenarios projected for the period of the 2050s (Ciais *et al.*, 2005) For the representative year 2045, the projected annual means for air temperature and precipitation at Saint-Genès-Champanelle, France, were 10.9 °C and 770 mm (corresponding to +2.3 °C and –33 mm, respectively, compared with the 1990–2009 means). Therefore, the control treatment consisted of warmer and drier climatic conditions than the long-term climatic conditions occurring at Saint-Genès-Champanelle. The atmospheric CO_2 concentration projected for the 2050s under the A2 scenario is 520 ppm compared with the ambient CO_2 concentration (aCO_2) of 390 ppm measured at the Mauna Loa Observatory (Hawaii) in 2010.

Forty-eight monoliths (1 m^2 each) of intact soil and their resident plant communities were excavated in June 2009, down to the bedrock at 0.6 m depth, from an extensively managed upland semi-natural grassland site (Redon, 45°43′N, 03°01′E, 800 m a.s.l.) near Saint-Genès-Champanelle in the French Massif Central. The average botanical composition of plant communities was initially dominated by C_3 perennial grasses (60%), legumes (35%), and forbs (5%). The grasses *D. glomerata* and *H. lanatus* were present in all monoliths and constituted 37% and 14%, respectively, of the initial above-ground community biomass. The soil in this site is a Cambisol (59.5% sand, 19.2% silt, 21.4% clay, pH H_2O 5.9).

The excavated monoliths were brought to Clermont-Ferrand INRA research station (45°46′N, 03°08′E, 350 m a.s.l., long-term 1980–2010 mean annual temperature 11.4 °C) for temperature acclimation until September 2009, where they received ambient precipitation and irrigation to maintain soil water content (SWC) near 80% of field capacity. They were then transported to Montpellier, and four monoliths representative of the species composition of the grassland were inserted in each of the 12 Ecotron macrocosms, where they acclimated, at aCO_2, from April 2010 to early March 2011 to the climatic conditions of the representative year 2045. The Ecotron climate-regulation system tracked the hourly means of air temperature, air humidity, and daily precipitation projected by the model.

From mid-March 2011 to the end of the experiment, six macrocosms randomly selected out of the 12 were subjected to eCO_2 (520 ppm) while the other six were subjected to aCO_2 (390 ppm) (Fig. 1). ECE included a reduction in precipitation by 50% during 4 weeks in midsummer (25 June to 21 July: date D1) followed by 2 weeks (22 July to 4 August: date D2) with no irrigation and a concomitant heat wave (+3.4 °C compared with the 2050s average) (Fig. 1). This increase in air temperature corresponded to 7.1 °C above the 2000–2009 average for the same period, a value above the average of the 14 consecutive hottest days of the exceptional heat wave in summer 2003. This ECE, mimicking the strongest of such events projected by the downscaled ARPEGEv4 model over 2040–2060, was applied to three randomly selected macrocosms out of the six at each CO_2 concentration. After the ECE, from 5 to 31 August, irrigation was progressively increased in the treatment with ECE to obtain the same cumulative precipitation as in the control treatment (Con) referring to the 2050s climatic conditions without ECE. After the ECE and until the end of the experiment on 3 November, the macrocosms subjected to each CO_2 treatment had the same climatic conditions, replicating the downscaled model projections for the representative year 2045. Each of the four experimental treatments (390 Con, 390 ECE, 520 Con, 520 ECE) was replicated three times. For further details on the experimental conditions, see Roy *et al.* (2016).

Plant community-level measurements

SWC was continuously measured at three soil depths (7, 20, and 50 cm) with TDR probes (IMKO, Ettlingen, Germany) and averaged across soil depths to assess relative SWC (RSWC) at each sampling date. The field capacity of the soil (SWC_{fc}) was 32.90% and the minimum soil moisture (SWC_{min}) at the end of the ECE, when all plants were senescent, was 8.16%. RSWC was calculated as the fraction of soil moisture available for plants with this equation: $RSWC=(SWC-SWC_{min})/(SWC_{fc}-SWC_{min})$. The vegetation was cut at 5 cm height on 14 March, 26 April, 9 June, and 3 November, to mimic mown grassland. After harvest, samples of the last three cuts were frozen at –18 °C, then defrosted, sorted by species, and oven-dried at 60 °C for 48 h to determine specific level above-ground biomass. The March cut was excluded from the analyses as it represented biomass production from the previous autumn, before the onset of CO_2 treatment. For each species and each of the four treatments, resilience to ECE was estimated by the biomass resilience index (RESIL), calculated as the ratio of above-ground biomass harvested after the ECE (November cut) to above-ground biomass harvested before the ECE (mean of April and June cuts). A RESIL value of 1 corresponds to full recovery of species biomass. This index was chosen from among others (Ingrisch and Bahn, 2018) because the biomass data harvested in November integrate ECE resistance and recovery periods. This index allows comparison of the treatments because it is calculated for each replicate macrocosm.

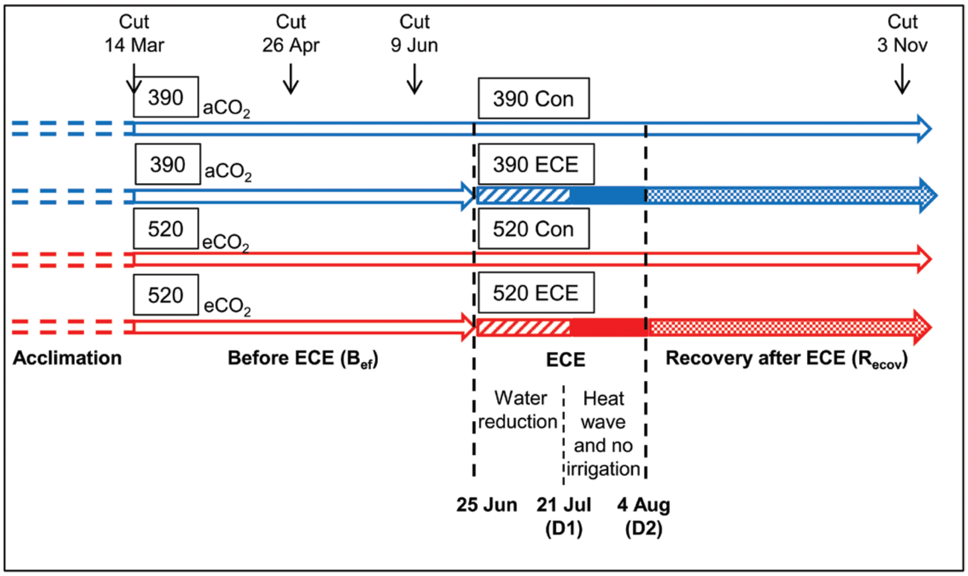

Fig. 1. Experimental design and time course of the experiment. After a 1-year acclimation period, the vegetation was cut at 5 cm height (14 March) and the monoliths were submitted to four climatic scenarios: ambient (aCO_2: 390 ppm, blue) or elevated (eCO_2: 520 ppm, red) atmospheric CO_2 concentration, combined with an extreme climatic event (390 ECE and 520 ECE) or without ECE (390 Con and 520 Con). The ECE included a reduction in precipitation by 50% during 4 weeks in midsummer (25 June to 21 July: D1) followed by 2 weeks (22 July to 4 August: D2) with no irrigation and a concomitant heat wave (+3.4 °C compared with the projected 2050s average). Thereafter, the precipitation was gradually increased. The vegetation was cut at 5 cm height again on 26 April, 9 June, and 3 November. Leaf meristematic tissues were sampled at D1 (21 July) and D2 (4 August).

Plant traits

Before the onset of ECE (21 June) to 3 weeks after the ECE (22 September), lamina length was measured twice a week on the fastest-growing leaves of four tillers per species (*D. glomerata* and *H. lanatus*) and per macrocosm to analyse the dynamics of leaf elongation rates. The mean leaf elongation rates measured during the ECE ($\mathrm{LER_{ECE}}$) and the maximum leaf elongation rates measured during the recovery period (LERr) were analysed. Pre-dawn leaf water potential was measured on three detached green laminae from each species in each monolith on 17 June, 21 July (D1), and 4 August (D2) (Scholander pressure chamber, model 1000, PMS Instrument Company, Corvallis, OR, USA). On the same days, the relative water content (RWC) of these laminae was measured by weighing lamina tissue before and after rehydration overnight at 4 °C and after drying at 80 °C for 24 h.

Leaf and leaf meristem traits

For each species, leaves collected during the cuts on 26 April, 9 June, and 3 November and those used for water potential measurements were oven-dried at 60 °C for 48 h and then ball milled (MM200, Retsch, Germany). Leaf nitrogen content was analysed in the Isotopic platform of INRA Nancy (Isoprime100, IsoPrime, Manchester, UK). At dates D1 and D2, measurements were carried out on the leaf meristematic tissues (three tillers for each macrocosm), which are the organs that survive the longest in perennial grasses. From the fraction containing the lowest 20 mm of the tillers, the enclosed bases of immature leaf meristems were dissected out and split out into two subsamples. One was immediately weighed and then dried at 80 °C for 24 h to determine its water content. The other fresh subsample was used to measure electrolyte leakage (Volaire and Thomas, 1995) from the cells (seed analyser G2000, Wavefront Inc., Ann Arbor, MI, USA) and calculate the coefficient of membrane stability (CMS).

For the WSC analysis, on the same dates, three other subsamples of fresh leaf meristems per species and monolith were quickly weighed, dropped into liquid nitrogen and stored at −80 °C before freeze-drying at −100 °C for 48 h. Finely powdered samples (30–50 mg) were extracted in 80% ethanol and purified in mini-columns (Mobicols, MoBITec, Göttingen, Germany) with ion-exchange resins (Amiard *et al.*, 2003). WSCs (mg $\mathrm{g^{-1}}$) were analysed by HPLC on a cation exchange column (Sugar-PAK, 300×6.5 mm, Waters Corporation, Milford, MA, USA), eluted at 0.5 ml $\mathrm{min^{-1}}$ and 85 °C with 0.1 mM Ca-EDTA in water, and quantified using a refractive index detector (2410 Differential Refractometer, Waters Corporation, Milford, MA, USA). External standards used to quantify carbohydrates were glucose, fructose, sucrose, and *Cichorium intybus* inulin (Sigma-Aldrich, MO, USA).

Carbohydrates in the assay mixture were analysed by high-performance anion exchange chromatography and pulsed amperometric detection (HPAEC-PAD ICS-3000, Dionex, CA, USA) equipped with a CarboPac PA1 anion-exchange column (4×250 mm) with elution at 1 ml $\mathrm{min^{-1}}$ with 150 mM NaOH and an increasing concentration of sodium acetate: 0 mM (0–6 min), 50 mM (6–12 min), 100 mM (12–18 min), 175 mM (18–19 min), 250 mM (19–30 min), 425 mM (30–60 min). Fructan type (levan versus inulin) and DP were identified by comparison with standards of commercial fructans (1-kestotriose, 1,1-kestotetraose; Megazyme, Ireland), purified fructans (6-kestotriose; kind gifts of Professor I. Izuka, Hyogo, Japan) and inulin extracted from *Taraxacum* spp. Comparison of standards with *D. glomerata* and *H. lanatus* extracts revealed that both species accumulated β2,6 linked fructan (levans), in accordance with the literature (Chatterton *et al.*, 1993; Bonnett *et al.*, 1997). Owing to the lack of commercially available analytical standards for fructans, absolute quantification of each peak was not possible. Instead, the relative amounts of the same DP were compared for each sample. The peak area of each DP of β2,6 fructan polymer (using the major peak when multiple peaks were present) from DP3 to DP65 was used to calculate the relative area of each peak as a percentage of the sum of DP3 to DP65 peak areas. As the amperometric response declines with increased chain length, it was not possible to compare the relative area of fructans differing in DP. The relative area of each peak (or group of peaks) expressed as a percentage of total peak area was used to analyse the treatment effects for each peak (or range of DPs). For analysis of the correlations between traits across species (Supplementary Table S1 at *JXB* online), the area percentage was weighted by the total fructan content in each sample.

Statistical analysis

Statistical analyses were carried out with R software (RStudio Team, 2015). Data were transformed when necessary (log, inverse, square, power 4, 5, or 6, boxcox) before analysis of variance (ANOVA) with the 'lmtest' package to conform to the assumptions of normality (Shapiro–Wilk test), homogeneity of variances (Bartlett test), and independence (Durbin–Watson test) or normality (Shapiro–Wilk test) of residues. For data for which the assumptions of normality, homogeneity of variances, and/or normality of residues were not obtained, analyses were carried out with the non-parametric Kruskal–Wallis test. For each trait, the factors species (*D. glomerata, H. lanatus*), $\mathrm{CO_2}$ concentration (390, 520 ppm), climatic condition (Con, ECE), and date (D1, D2), as well as all of their interactions, were explored. Relationships between cell membrane stability and WSC, and relationships between resilience and plant traits, were analysed with non-parametric Spearman correlation tests.

Results

Responses of above-ground biomass

For both species, above-ground biomass in the spring before the ECE was not affected by the $\mathrm{CO_2}$ concentration and was approximately 2-fold greater for *H. lanatus* than for *D. glomerata* (P=0.007; Table 1, Fig. 2A, B). In the autumn after the ECE (recovery period), no difference in biomass production was found between the species; the biomass of both species was slightly higher under $\mathrm{eCO_2}$ (P=0.077), with a significant interaction between species and ECE (P=0.01; Table 1, Fig. 2A, B). As a result, the RESIL for control plants of *D. glomerata* was around 1.1 (±0.6) in both $\mathrm{CO_2}$ treatments, and equal to 1.4 (±0.7) and 1.7 (±0.8) after the ECE under $\mathrm{aCO_2}$ and $\mathrm{eCO_2}$, respectively (Fig. 2C). By contrast, the RESIL was close to zero after the ECE for *H. lanatus* but it was 2-fold higher (0.75±0.47) for the control plants under $\mathrm{eCO_2}$ than under $\mathrm{aCO_2}$ (0.34±0.16) (Fig. 2D).

Responses at leaf level

During the ECE, the mean leaf elongation rate ($\mathrm{LER_{ECE}}$) was significantly (P<0.0001) affected by all fixed factors (species, $\mathrm{CO_2}$, ECE) and with significant interactions between factors (Table 1). Across all treatments, $\mathrm{LER_{ECE}}$ was 3- to 5-fold higher for *D. glomerata* than for *H. lanatus*, for which the variability of data was also greater (Fig. 3A, C). The pattern of $\mathrm{LER_{ECE}}$ response was similar for both species, with higher rates under $\mathrm{eCO_2}$, a decline during the ECE, and a final cessation of elongation for all stress treatments under less than 20% of RSWC. At that time, all leaves were mostly senescent for all plants of the swards under stress (Roy *et al.*, 2016). As the stress progressed, the leaf water potential declined down to −7 MPa in both species (Table 2). The leaf RWC was significantly higher in *D. glomerata* (P=0.03), although a $\mathrm{CO_2}$×ECE interaction was significant for both leaf water potential and RWC (Table 1). Leaf nitrogen content was on average 20% higher in *D. glomerata* than in *H. lanatus*, and declined during the ECE for both species, but increased at the lowest RSWC,

Table 1. *Effect of species (*D. glomerata, H. lanatus*), CO_2 concentration (390, 520 ppm), climatic condition (Clim) (Con, ECE), date (D1, D2) and their interactions, based on ANOVA, on above-ground biomass (BIOM), resilience index (RESIL), mean leaf elongation rate during ECE (LER_{ECE}), maximum leaf elongation rate during the recovery period (LERr), leaf pre-dawn water potential (POT), leaf relative water content (RWC), and leaf nitrogen content (LNC)*

Period	Variable	Species	CO_2	Clim	Species× CO_2	Species× Clim	CO_2× Clim	Species× CO_2×Clim	Date	Date× Species	Date× CO_2	Date× Clim
Before ECE	BIOM	0.007	NS		NS							
During ECE	LER_{ECE}	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0037	NS				
	POT	<0.0001	NS	<0.0001	NS	<0.0001	0.0188	NS	<0.0001	NS	NS	<0.0001
	RWC	0.03	NS	<0.0001	NS	NS	0.019	NS	<0.0001	NS	0.006	<0.0001
	LNC	<0.0001	<0.0001	NS	0.032	NS	NS	NS	NS	NS	NS	NS
Recovery after ECE	LERr	<0.0001	<0.0001	<0.0001	<0.0002	<0.0001	0.0042	<0.0001				
	LNC	0.0101	0.0100	<0.0001	NS	NS	NS	NS				
	BIOM	NS	0.077	NS	NS	0.010	NS	NS				
	RESIL	0.0047	NS	NS	NS	NS	NS	NS				

P values are shown when <0.05. NS, Not significant. Three periods are considered: before ECE, during ECE, and during recovery after ECE (see Fig. 1)

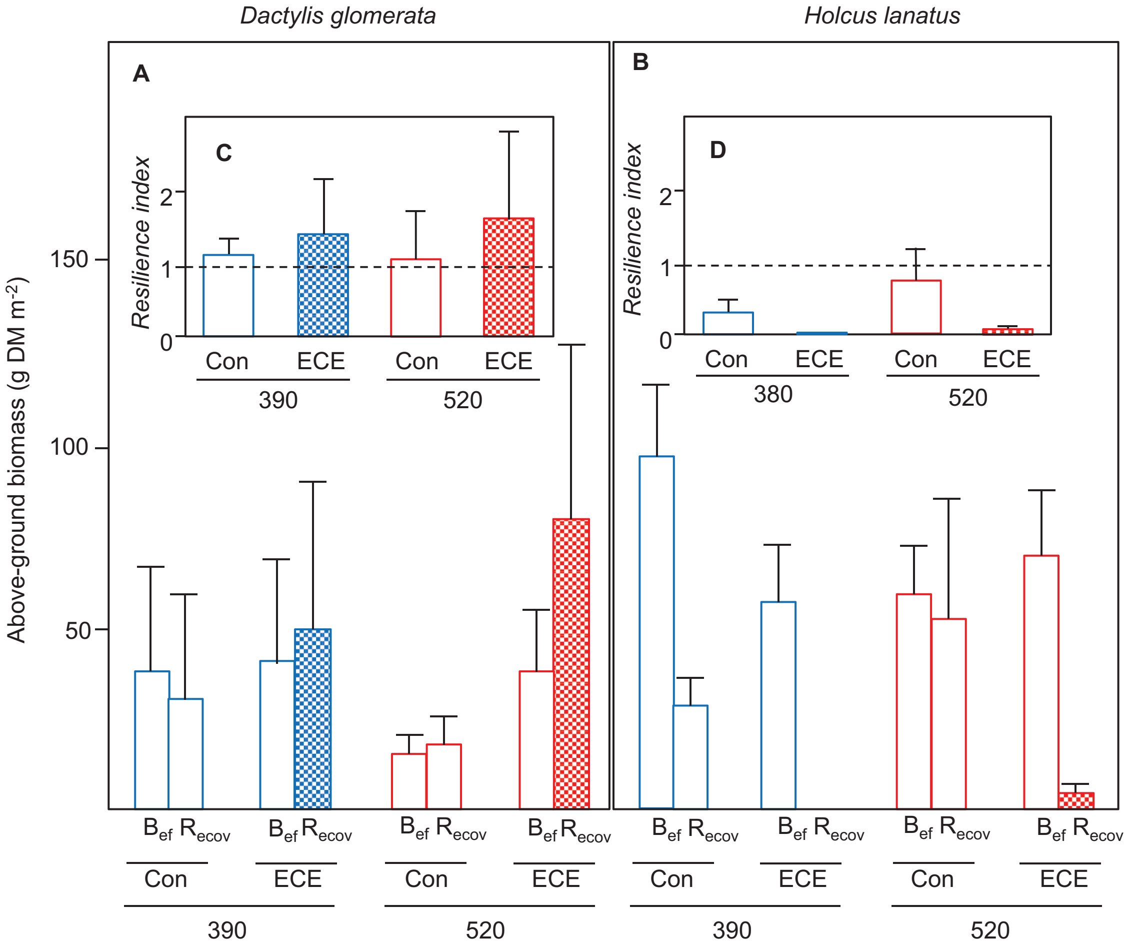

Fig. 2. Above-ground biomass (A, B) and resilience index (C, D) of two perennial grass species, *D. glomerata* (A, C) and *H. lanatus* (B, D) under four climatic scenarios: 390 (blue) or 520 (red) ppm atmospheric CO_2 concentration, combined with the ECE or without ECE (Con). Above-ground biomass was the biomass harvested above 5 cm height before the ECE (B_{ef}; mean of two cuts on 26 April and 9 June) and after the ECE at the end of the recovery period (R_{ecov}; 3 November cut). For the recovery period, the absence of a bar for *H. lanatus* grown at 390 ECE coincides with mortality. The resilience index was calculated by the R_{ecov} to B_{ef} above-ground biomass ratio; A value of 1 corresponds to full recovery of species biomass. Mean ±SD are shown (*n*=3).

with a significantly lower nitrogen content in *H. lanatus* at eCO_2 (Supplementary Fig. S1).

During the recovery period, all treatments and interactions significantly affected the LERr (Table 1). Although some

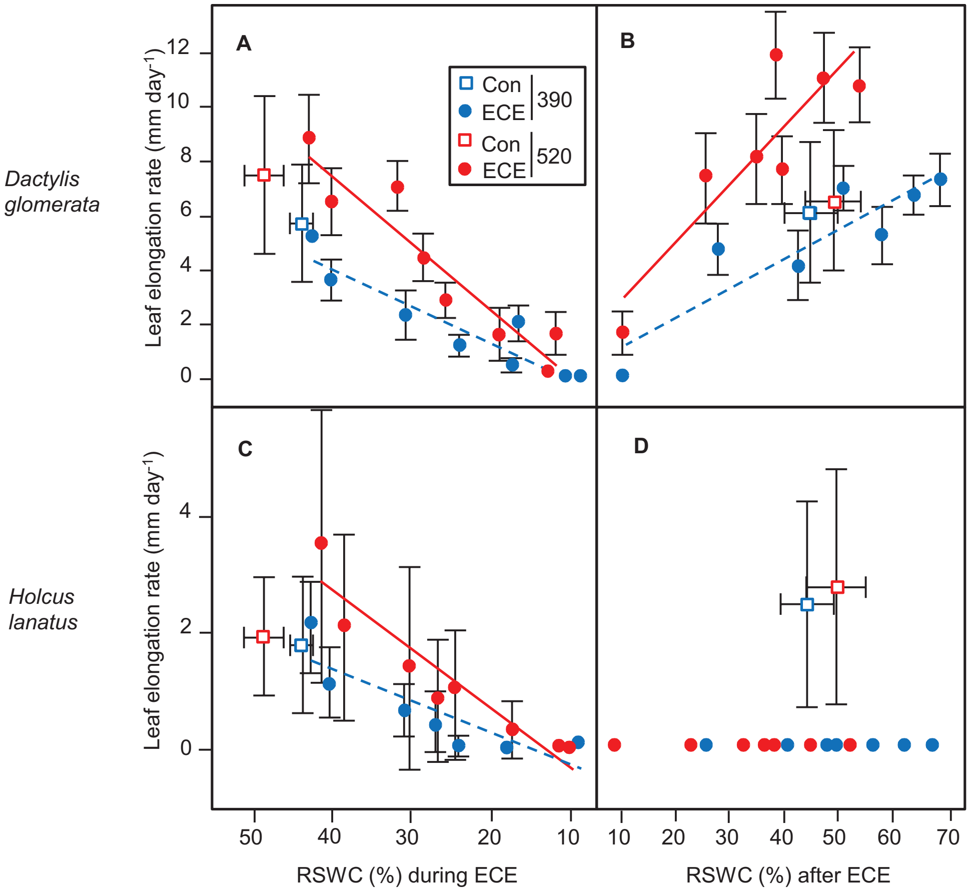

Fig. 3. Leaf elongation rate during the ECE (A, C) and during the recovery period after the ECE (B, D) as a function of the relative soil water content (RSWC) of two perennial grass species, *D. glomerata* (A, B) and *H. lanatus* (C, D). Blue and red symbols correspond to 390 ppm and 520 ppm atmospheric CO_2 concentration, respectively. Squares correspond to control treatments (390 Con and 520 Con), for which only mean values of the three periods are shown. Blue triangles and dotted blue lines correspond to ECE treatment at 390 ppm CO_2. Red triangles and solid red lines correspond to ECE treatment at 520 ppm CO_2. Mean ±SD are shown (*n*=3). For 390 Con and 520 Con, horizontal (RSWC) and vertical (LER) SD are shown.

Table 2. *Pre-dawn leaf water potential and lamina relative water content (RWC) of* D. glomerata *and* H. lanatus *under four climatic scenarios*

	390 ppm				**520 ppm**			
	Control	**ECE**			**Control**	**ECE**		
	3 dates	**Before ECE**	**D1**	**D2**	**3 dates**	**Before ECE**	**D1**	**D2**
D. glomerata								
Leaf water potential (–MPa)	0.9±0.3	1.1±0.3	3.3±0.9	6.7±0.6	0.9±0.2	0.9±0.3	3.1±0.2	7±0
Lamina RWC (%)	90.9±3.4	80.2±2.7	53.5±6.1	38.2±0.3	84.1±8.8	84.3±1.7	62.3±13.4	39.7±6.7
H. lanatus								
Leaf water potential (–MPa)	1.1±0.3	1.3±0.02	3.6±0.6	7.00±0	1.2±0.1	1.4±0.4	2±0.1	7±0
Lamina RWC (%)	84.3±7.4	81.9±5.3	65.2±7.3	10.8±5.9	83.8±5.4	79.8±5.0	65.7±16.7	43.4±18.9

Climatic scenarios were two atmospheric CO_2 concentrations (390, 520 ppm) with or without an ECE at three dates (before ECE, 17 June; during ECE, D1; end of ECE, D2; see Fig. 1). For the control treatment, average values of the three dates are shown. Data are presented as mean ±SD (*n*=3)

H. lanatus plants in the macrocosms recovered, none of the plants of this species sampled for LERr measurements recovered after the ECE (Fig. 3D). The LERr of *D. glomerata* reached that of control plants at aCO_2 for a RSWC greater than 50% (Fig. 3B). In addition, the recovery of *D. glomerata* was greatly enhanced under eCO_2, since its LERr reached up to 12 mm day^{-1}, that is, 2-fold more than the control plants (Fig. 3B). In parallel, leaf nitrogen content more than doubled during the recovery period for both species (Supplementary Fig. S1).

Responses at leaf meristem level

The water content and the CMS of meristematic tissues were not affected by CO_2 concentration but were significantly reduced in both species by the ECE treatment (Table 3, Fig. 4).

Table 3. *Effect of species, CO_2 concentration (390, 520 ppm), climatic condition (Clim) (Con, ECE), date (D1, D2), and their interactions (based on ANOVA) on water content, cell membrane stability (CMS), soluble carbohydrate content, total fructans:sucrose ratio, and low DP:sucrose ratio in leaf meristems of* D. glomerata *and* H. lanatus

Variable		**Species**	**CO_2**	**Clim**	**Species× CO_2**	**Species× Clim**	**CO_2× Clim**	**Species× CO_2xClim**	**Date**	**Date× Species**	**Date× CO_2**	**Date× Clim**
	Water content	0.004	NS	<0.001	NS	NS	0.050	NS	<0.001	NS	NS	<0.001
	CMS	NS	NS	<0.001	NS	0.009	NS	NS	0.003	NS	NS	<0.001
Soluble carbohydrate content (mg g DM^{-1})	Fructans	<0.001	0.042	NS	NS	NS	NS	NS	<0.001	NS	NS	NS
	Sucrose	<0.001	NS	<0.001	NS	NS	0.038	NS	NS	NS	NS	<0.001
	Glucose	0.027	NS	NS	NS	<0.001	NS	NS	0.004	NS	NS	NS
	Fructose	NS	NS	NS	NS	0.004	NS	NS	<0.001	NS	NS	NS
Fructan composition (relative content, %)	Low DP	<0.001	NS	NS	NS	0.004	NS	NS	NS	NS	0.018	NS
	Medium DP	NR	NS	NS	NR	NR	NS	NR	<0.001	NR	NS	NS
	High DP	NR	NS	NS	NR	NR	NS	NR	0.012	NR	NS	NS
Fructans:sucrose ratios	Total fructans:sucrose	<0.001	NS	<0.001	NS	NS	NS	NS	NS	NS	NS	<0.001
	Low DP:sucrose	0.001	NS	0.002	–	–	–	–	NS	–	–	–
	Medium DP:sucrose	NR	NS	<0.001	NR	NR	NS	NR	NS	NR	NS	<0.001
	High DP:sucrose	NR	NS	NS	NR	NR	NS	NR	NS	NR	NS	0.004
Fructans:fructans ratios	Low DP:Medium DP	NR	NS	NS	NR	NR	–	NR	NS	NR	–	–
	Low DP:High DP	NR	NS	NS	NR	NR	NS	NR	0.0037	NR	0.019	NS
	Medium DP:High DP	NR	NS	NS	NR	NR	–	NR	NS	NR	–	–

For medium and high DP composition, medium DP:sucrose, high DP:sucrose and fructans:fructans ratios, the effect of species was not relevant (NR) since groups of medium and high DP were not similar in the two species. For the ratios of low DP:sucrose; low DP:medium DP, and medium DP:high DP, the effects of CO_2, Clim, and date were analysed independently by non-parametric Kruskal–Wallis tests. *P* values are shown when <0.05. NS, Non-significant effect. –, Conditions for analysis not met.

In both species, fructans were the main WSCs in leaf meristematic tissues (Fig. 4, Supplementary Fig. S2). Levels of fructans, sucrose, and glucose, but not fructose, significantly differed between the species (Table 3). Overall, fructan content was ~30% higher in *D. glomerata* (mean 526 mg g DM^{-1}) than in *H. lanatus* (mean 357 mg g DM^{-1}), while sucrose content was 3- to 5-fold lower in *D. glomerata* (mean 16 mg g DM^{-1}) than in *H. lanatus* (mean 53 mg g DM^{-1}) (Table 3, Fig. 4). Fructan content was higher under eCO_2 (Table 3, Fig. 4E, F). Moreover, fructan content increased between D1 and D2 in ECE-treated and control plants of *D. glomerata* (P=0.027; *t*-test) and *H. lanatus* (P=0.026; *t*-test) (Fig. 4E, F). Sucrose content was affected by the ECE, with interactions with CO_2 concentration and date (Table 3, Fig. 4G, H). The ECE increased sucrose content at D2, with a bigger effect under aCO_2 than under eCO_2 (Fig. 4G, H).

The analysis of fructan composition (Fig. 5) showed that fructans consisted of a mixture of oligo- and polysaccharides of mainly β(2,6) fructans (levans) in both species. In *D. glomerata*, fructans up to DP65 were identified, while in *H. lanatus* the highest detected DP was only 45. The pattern of fructan DP distribution varied between D1 and D2 and differed among species (Figs 6 and 7; Table 3). In *D. glomerata*, the relative abundance of DP9–24 increased while that of DP40–55 decreased (Fig. 6A, B), so that their ratio increased (Fig. 6C). Conversely, in *H. lanatus*, the relative abundance of DP9–12 declined while that of DP16–30 increased (Fig. 7A, B), so that their ratio decreased (Fig. 7C). Fructans were divided into three groups corresponding to low, medium, and high DP. For both species, low DP corresponds to the sum of DP3 and DP4. Medium DP corresponds to DP9–24 for *D. glomerata* and DP9–12 for *H. lanatus*. High DP corresponds to DP40–55 for *D. glomerata* and DP18–30 for *H. lanatus*. Other fructan DPs were not considered since they were not affected by the environmental factors (CO_2 concentration, climatic condition, date) (Figs 6 and 7). The relative abundance of the three DP groups of fructans was not affected by eCO_2 (Table 3, Supplementary Fig. S3).

For each species, correlations between CMS and carbohydrate data in leaf meristems measured at D1 and D2 were investigated (Table 4). In both species, CMS was not correlated to fructan content or fructan composition (relative content of low- and high-DP fructans). In *H. lanatus*, CMS was negatively correlated to relative content of medium-DP fructans. In both species, CMS was lowest when the sucrose content was highest (Fig. 8A, B), and CMS was negatively correlated to sucrose content in *H. lanatus* (Table 4). CMS was negatively correlated to hexose contents in *D. glomerata*, and positively correlated to hexose contents in *H. lanatus* (Table 4). In *H. lanatus*, CMS was correlated with the low-DP fructans to sucrose ratio and with sucrose content (r=0.70 and –0.65, respectively; Table 4, Fig. 8D).

For the analysis of correlations across species between resilience to the ECE and plant traits, the traits related to medium- and high-DP fructans were not considered, since the DP groups differed according to the species (Figs 6 and 7). The best predictors of resilience were the fructans to sucrose ratios, especially the low-DP fructans to sucrose ratio in leaf meristems at D1 and D2 (Supplementary Table S1, Fig. 9).

Discussion

This study explored the response of two coexisting perennial grass species growing in a community of a temperate semi-natural grassland grown under the predicted climatic

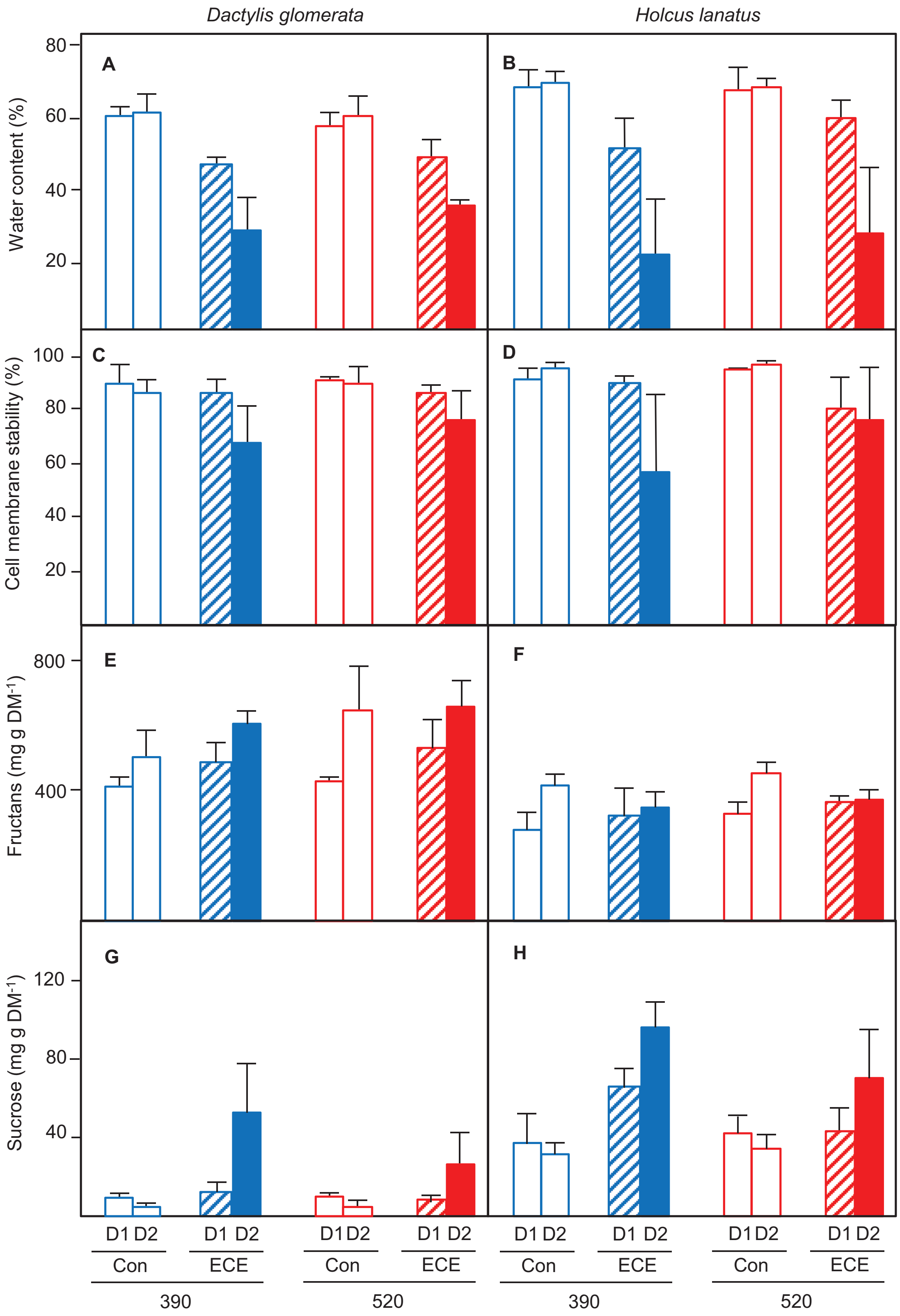

Fig. 4. Water content (A, B), cell membrane stability (C, D), fructan content (E, F), and sucrose content (G, H) in leaf meristems of two perennial grass species, *D. glomerata* (A, C, E, G) and *H. lanatus* (B, D, F, H) under four climatic scenarios: 390 (blue) or 520 (red) ppm atmospheric CO_2 concentration, combined with the ECE treatment (ECE) or without ECE (Con). Leaf meristematic tissues of the two species were sampled at D1 (21 July) and D2 (4 August). Mean ±SD are shown (*n*=3).

conditions of the 2050s, subjected or not subjected to an ECE including extreme drought and a heat wave, under ambient or elevated CO_2 concentrations. The resistance during the ECE and recovery after the ECE were high for *D. glomerata* and lower for *H. lanatus*. These results confirm that most native grasslands are likely to contain plants with a high diversity

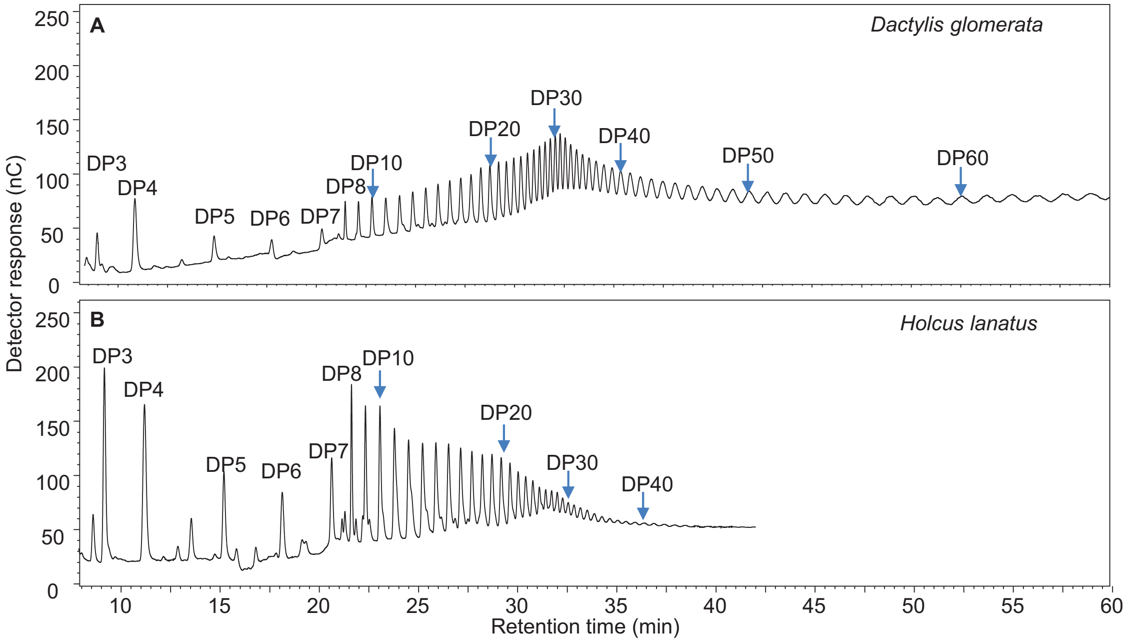

Fig. 5. Distribution of the fructans according to their degree of polymerization (DP). HPAEC-PAD chromatogram profiles of water-soluble carbohydrates in leaf meristems of two perennial grass species, *D. glomerata* (A) and *H. lanatus* (B). DP for levans [β(2,6) linked fructans] are indicated above the corresponding peak.

of drought resistance (Craine *et al.*, 2013) and also drought survival after severe water deficit (Pérez-Ramos *et al.*, 2013; Zwicke *et al.*, 2015). Contrary to our hypothesis, we found no significant mitigating effect of eCO_2 on the mortality of *H. lanatus*. However, eCO_2 had a positive effect on both resistance and recovery of *D. glomerata*. These results are discussed in light of the water, nitrogen, and carbon status response of the plants to the treatments.

Elevated CO_2 increases the drought resistance of H. lanatus *and* D. glomerata *during moderate drought*

Both grass species had higher leaf growth and leaf RWC under a combination of eCO_2 and moderate drought during the first stage of ECE. As shown at community level, these positive effects should have slowed leaf senescence and maintained photosynthesis for a longer period during drought than under aCO_2 (Roy *et al.*, 2016), leading to the higher content of fructans in leaf meristems. This confirms the importance of improved water relations under eCO_2, which to some degree sustain photosynthesis in dry periods (Albert *et al.*, 2011*b*). In addition, as leaf N content was significantly higher in *D. glomerata* than in *H. lanatus*, it may be hypothesized that the photosynthetic activity of *D. glomerata* is higher than that of *H. lanatus*, especially under eCO_2 (CO_2×species interaction: Table 1; Harmens *et al.*, 2000). Altogether, our results underline and confirm that eCO_2 alleviates the effects of drought stress by conservation of water (Morgan *et al.*, 2001, 2011; Robredo *et al.*, 2007; Holtum and Winter, 2010), higher carbon fixation, and higher fructan accumulation; thus, eCO_2 contributed to increased drought resistance. Previous studies have shown that the level of WSC accumulation in response to eCO_2 largely depended on N availability in the leaves (Dumont *et al.*, 2015). Here, the relatively low WSC accumulation under eCO_2 may indicate that N availability was high enough to sustain leaf growth (Fig. 3), root growth (Roy *et al.*, 2016), and root exudation (Pendall *et al.*, 2004), which limited storage of WSCs in both species.

H. lanatus *and* D. glomerata *show contrasting ECE survival*

Although *H. lanatus* and *D. glomerata* are both C_3 perennial grasses co-occurring in temperate grassland with similar rooting depth (Pages and Picon-Cochard, 2014), they showed contrasting survival after the ECE. It was previously shown that although species with different growth habits varied in their responses to CO_2 and nitrogen, there was also substantial variation in responses among species within groups (Reich *et al.*, 2001). In the present study, almost all *H. lanatus* plants died, whereas all *D. glomerata* survived. This stark contrast between a 'loser' and a 'winner' species (Dukes, 2007) strongly affected the botanical composition of the grassland macrocosms following the treatments (data not shown). As the grassland macrocosms had a realistic soil depth of 0.6 m, it can be assumed that both species had a rooting depth able to explore the full soil profile and therefore that their potential water uptake was comparable under drought. It has been suggested that long-term responsiveness to rising CO_2 concentrations differs between slow- and fast-growing plants (Ali *et al.*, 2013). Our results also tend to suggest that growth patterns, with a much greater growth potential for *D. glomerata* than *H. lanatus*, may be associated with greater ECE survival, irrespective of the treatments. Although above-ground growth of both species was maintained for a similar period in the summer, the water status of leaves and meristems of *D. glomerata* was higher at some dates, especially under eCO_2. Altogether, the species with a greater acquisition strategy and higher growth rate (*D. glomerata*) survived better than the species with a more resource-conservative strategy

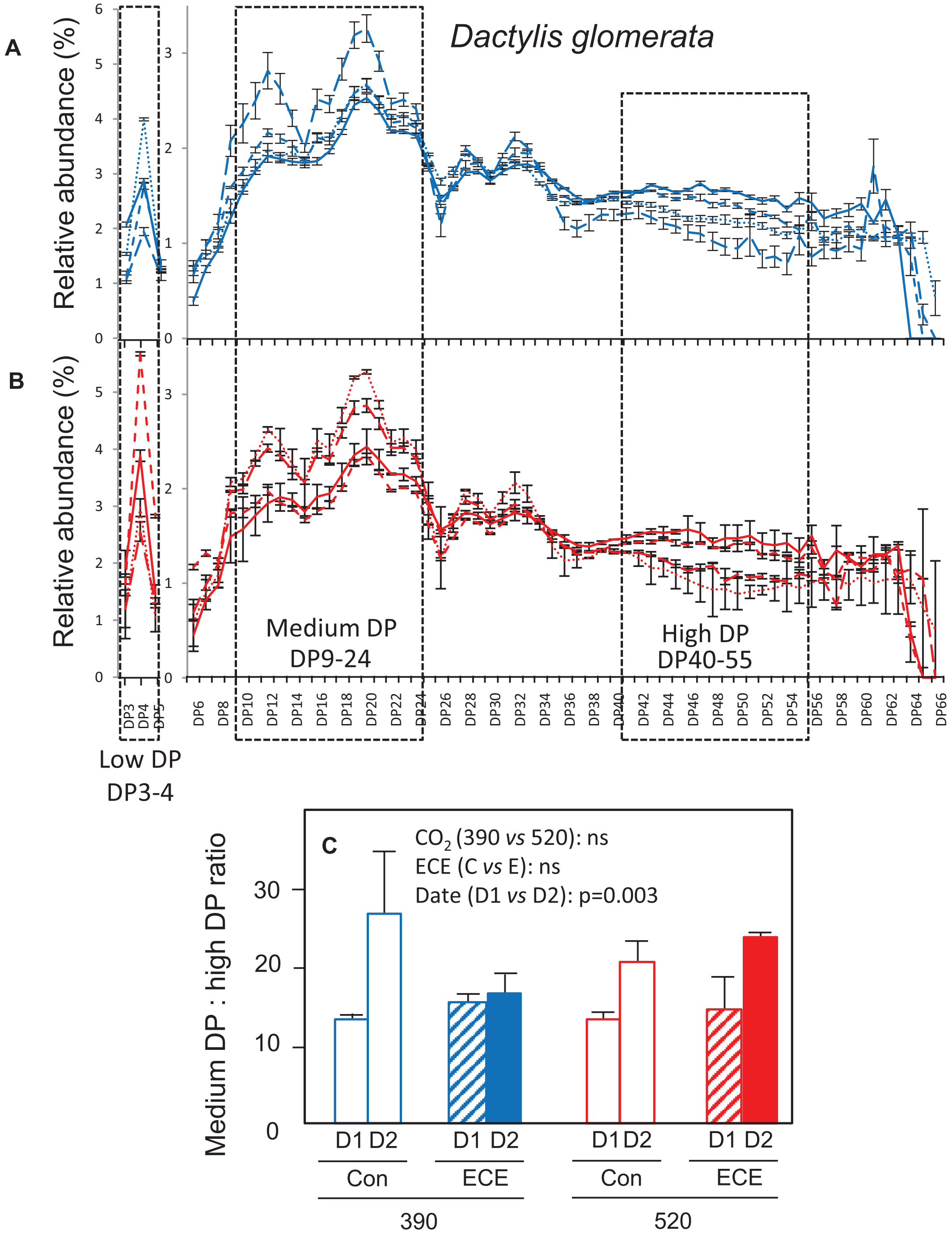

Fig. 6. Relative abundance of fructans based on their degree of polymerization (DP) (A, B) and ratio of medium- to high-DP fructans (C) in leaf meristems of *D. glomerata* under four climatic scenarios: 390 (A) or 520 (B) ppm atmospheric CO$_2$ concentration combined with the ECE treatment (ECE) or without ECE (Con). The dashed boxes include the two groups of fructans with medium and high DP that were the most altered by the treatments. Leaf meristematic tissues were sampled at D1 (21 July) and D2 (4 August). Mean ±SD are shown (*n*=3).

(*H. lanatus*). Assuming that this pattern for temperate ecotypes of perennial grasses can be generalized, it differs from the pattern found for Mediterranean ecotypes of perennial grasses such as *D. glomerata*, for which a reduction or cessation of leaf growth (summer dormancy) are the most efficient strategies to conserve soil water resources and therefore to survive severe drought (Volaire, 1995;Volaire and Norton, 2006).

Since the leaves were mostly senescent at the end of the ECE for all species present in the community (Roy *et al.*, 2016), plant survival relied on maintenance of the viability of leaf meristems (Volaire, 1995;Volaire *et al.*, 2014). Our study found major differences between the tested species in WSC accumulation in meristematic tissues of leaves. The stress-tolerant species *D. glomerata* accumulated a larger amount of fructans than the sensitive species *H. lanatus*, which in turn accumulated a larger amount of sucrose. These differences in WSC level and composition might be associated with the two species' differential survival and recovery after the ECE. In particular, the

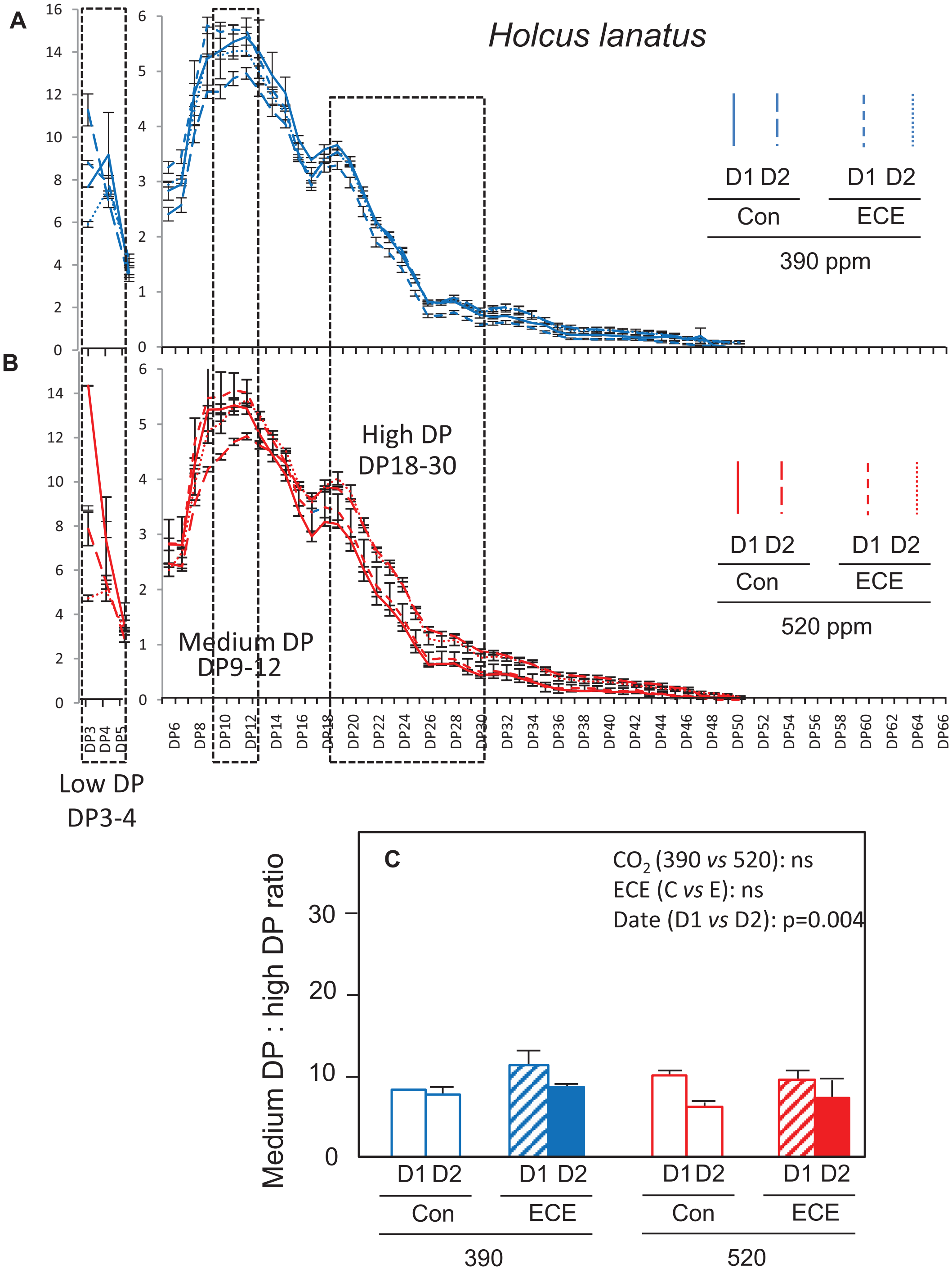

Fig. 7. Relative abundance of fructans based on their degree of polymerization (DP) (A, B) and ratio of medium- to high-DP fructans (C) in leaf meristems of *H. lanatus* under four climatic scenarios: 390 (A) or 520 (B) ppm atmospheric CO_2 concentration combined with the ECE treatment (ECE) or without ECE (Con). The dashed boxes include the two groups of fructans with medium and high DP that were the most altered by the treatments. Leaf meristematic tissues were sampled at D1 (21 July) and D2 (4 August). Mean and ±SD are shown (*n*=3).

level of sucrose, which accumulated to a much greater extent in the sensitive species, was highly and negatively correlated to the maintenance of cell stability of the meristems at the end of the ECE, confirming previous results on temperate ecotypes of herbaceous species (Zwicke *et al.*, 2015). The higher degree of polymerization of fructans in *D. glomerata* (up to DP65) could contribute to its greater survival compared with *H. lanatus*, which accumulated smaller fructans (up to DP45). Similar differences have been observed among the Asteraceae family, in which drought-resistant species (*Echinops ritro* and *Vigueira discolor*) accumulate fructans of higher DP (30–100) (Itaya *et al.*, 1997; Vergauwen *et al.*, 2003) than species with lower drought resistance (*Cichorium intybus*, *Helianthus tuberosus*; Van den Ende *et al.*, 1996; Marx *et al.*, 1997). It has been observed *in vitro* that highly polymerized fructans (from bacteria) had a greater protective effect against water stress than lower DP fructans (from

Table 4. *Spearman correlation coefficients of soluble carbohydrate traits against cell membrane stability (CMS) measured in leaf meristems of* D. glomerata *and* H. lanatus *at D1 and D2 (*n*=24 for each species)*

Plant traits		**Correlation with CMS**	
		D. glomerata	***H. lanatus***
Soluble carbohydrate content (mg g DM^{-1})	Fructans	–0.34 NS	0.27 NS
	Sucrose	–0.33 NS	**–0.65*****
	Glucose	–0.56**	0.56**
	Fructose	–0.49*	0.46*
Fructan composition (relative content, %)	Low DP	0.01 NS	0.31 NS
	Medium DP	–0.04 NS	–0.42*
	High DP	0.05 NS	0.19 NS
Fructans:sucrose ratios	Total fructans:sucrose	0.11 NS	**0.64*****
	Low DP:sucrose	0.36 NS	**0.70*****
	Medium DP:sucrose	0.37 NS	**–0.64*****
	High DP:sucrose	0.34 NS	0.57**
Fructans:fructans ratios	Low DP:Medium DP	0.01 NS	0.54**
	Low DP:High DP	0.13 NS	0.17 NS
	Medium DP:High DP	–0.03 NS	–0.27 NS

P*<0.05, ***P*<0.01, * *P*<0.001; high significant Spearman correlations (*P*<0.001) are in bold. NS, Not significant.

Cichorium) (Demel *et al.*, 1998), and that positive synergistic effects on membrane stabilization were obtained with a mixture of DP<7 and DP>7 fructans (from oat or rye) compared with either DP<7 or DP>7 fructans alone (Hincha *et al.*, 2007). In *D. glomerata*, the high range of DP (from DP3 to DP65) could lead to a better synergistic effect than in *H. lanatus*, with its smaller range of DP, and this could partly explain the difference in membrane stability between the two species. Fructans can act on membranes either directly, by inserting into the lipid headgroups (Livingston *et al.*, 2009), or indirectly, by reducing lipid peroxidation through ROS scavenging (Peshev *et al.*, 2013). In *D. glomerata*, the maintenance of CMS observed under ECE might be due to the accumulation of highly polymerized fructans.

According to current knowledge, levans are synthesized by sucrose:fructan 6-fructosyltransferase (6-SFT) and degraded by fructan 6-exohydrolase (6-FEH) (Vijn and Smeekens, 1999). 6-SFT catalyses the transfer of a fructosyl residue from sucrose to sucrose or from sucrose to fructans by forming a β(2,6) fructosyl-fructose linkage. 6-SFT allows both the initiation of levan synthesis and the elongation of the levan chain. In *H. lanatus*, the relative abundance of the different fructan polymers changed during the ECE, resulting in a more polymerized assemblage. Given that at the same time, total fructan content did not increase, the entry of carbon (in the form of the fructosyl residue from sucrose) into the fructan pool via 6-SFT activity for levan elongation was balanced by an exit of carbon via 6-FEH activity, which catalyses the release of fructose from fructans. This fructan trimming strategy may be associated with the low ECE survival of *H. lanatus*. In *D. glomerata*, the total fructan content increased during the ECE and the relative abundance of the different fructan polymers changed, resulting in a more depolymerized assemblage. This might be due to the fact that all newly synthesized fructans were of medium DP or that, concomitantly with fructan synthesis, the pre-existing high-DP fructans were depolymerized. Such a strategy combining fructan trimming and accumulation may have enhanced the ECE survival of *D. glomerata*. In plant species that accumulate inulin [fructan consisting of linear β(2,1) linked fructosyl units], fructan synthesis requires two enzymes, a sucrose:sucrose 1-fructosyltransferase for initiation and a fructan:fructan 1-fructosyltransferase for elongation (Van den Ende *et al.*, 1996; Vijn and Smeekens, 1999). To date, proteins with fructan:fructan 6-fructosyltransferase (6-FFT) activity have not been described in levan-accumulating plant species. If 6-FFT exists in *H. lanatus* and *D. glomerata*, the results obtained would be interpreted differently. Indeed, contrary to 6-SFT, which catalyses both the initiation and elongation of fructans, 6-FFT is responsible only for fructan elongation without concomitant entry of carbon into the fructan pool, the fructosyl donor being fructan instead of sucrose. Consequently, changes in the relative abundance of fructans could be supported by the induction of 6-FFT activity in *H. lanatus* and by the increase of 6-SFT, 6-FFT, and/or 6-FEH activity in *D. glomerata*. Further research is needed to unravel levan metabolism and its regulation in levan-accumulating grass species.

In addition, the WSC composition was associated with resilience. Indeed, the ratio of low-DP fructans to sucrose present in the leaf meristems during the ECE was the best predictor of resilience. Altogether, the results showed that a fine-tuning of the relative content and composition of WSCs is crucial for ECE survival. As previously shown in wheat (Joudi *et al.*, 2012; Zhang *et al.*, 2015; Hou *et al.*, 2018) and among carbohydrate-metabolizing enzymes, FEHs are key proteins for ECE survival, since they are involved in fructan size adjustment for cell protection during the ECE and fructan mobilization for carbon feeding of growing cells after the ECE. Comparison of a larger inter- and intra-specific range of populations of grasses should allow further exploration of the mechanistic relationships between drought resilience and fructan metabolism.

Elevated CO$_2$ enhances strong compensatory growth after stress for the species surviving ECE

During the ECE, the overall water use efficiency at the community level was greater under eCO$_2$ (Roy *et al.*, 2016), while leaf extension rate in water-stressed plants of both species were also enhanced, as found previously (Casella and Soussana, 1997; Drake *et al.*, 1997). In our study, and as hypothesized, the most striking result was the enhancement of growth recovery of *D. glomerata* after the ECE under eCO$_2$. Under aCO$_2$, no compensatory growth of this species was observed after the ECE. These results contradict previous studies showing compensatory growth after drought in herbaceous species at aCO$_2$ but not eCO$_2$ (Newton *et al.*, 1996; Clark *et al.*, 1999). This discrepancy may be due to the different nature and intensity of the stress to which plants were subjected, but also to the duration of acclimation to eCO$_2$. Compared with the moderate

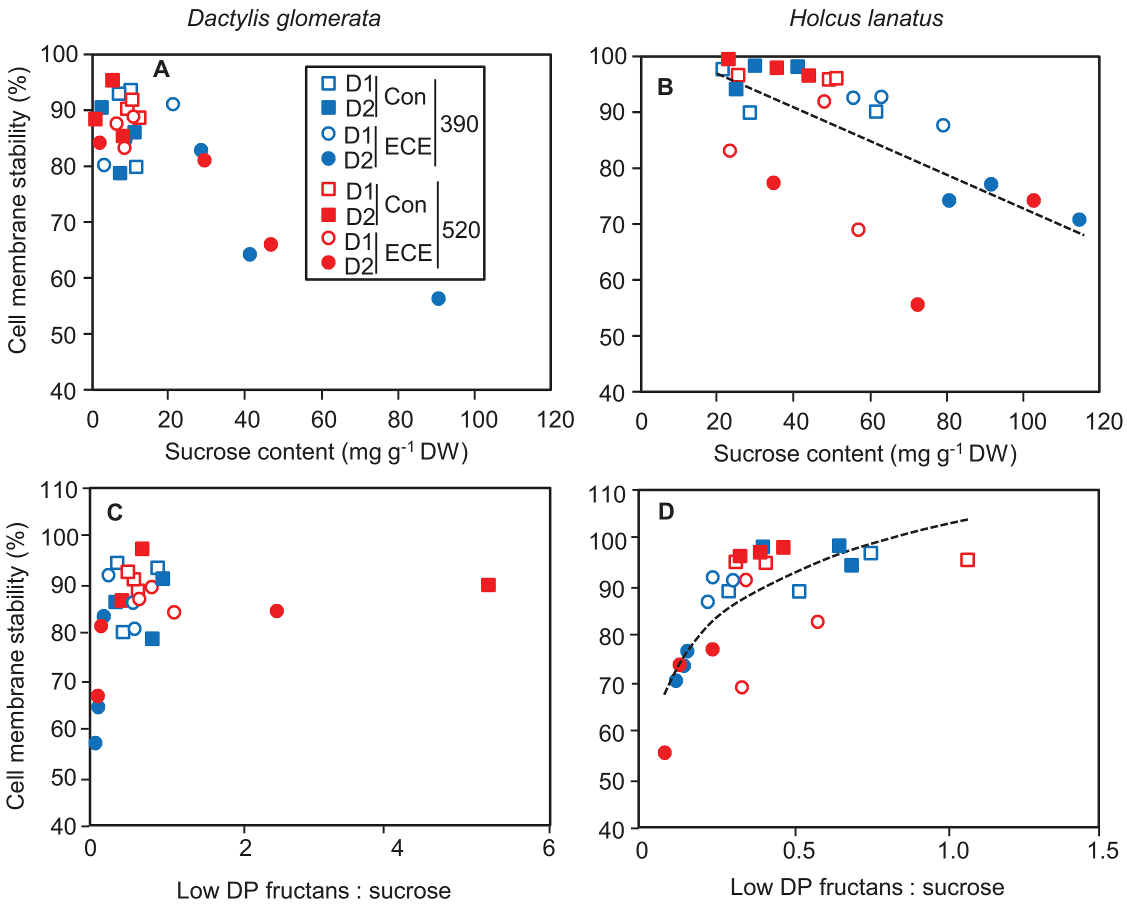

Fig. 8. Relationship between cell membrane stability and sucrose content (A, B) or the low-DP fructans to sucrose ratio (C, D) in *D. glomerata* (A, C) and *H. lanatus* (B, D). Leaf meristematic tissues of the two species were sampled at D1 (21 July) and D2 (4 August). Dashed lines indicate a linear relationship (in B) or a logarithmic relationship (in D) for a significant Spearman correlation.

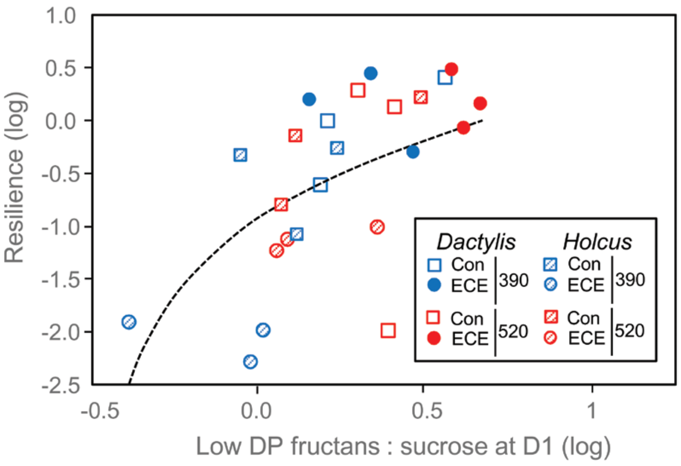

Fig. 9. Relationship between the resilience index of above-ground biomass of *D. glomerata* and *H. lanatus* and the ratio of low-DP fructans to sucrose level in leaf meristems measured at D1 (21 July). The dashed line indicates a logarithmic relationship for a significant Spearman correlation (*n*=2).

drought applied in previous studies, the severity of the ECE in the present study resulted in full above-ground senescence of all plants. It was only under eCO$_2$ that resource use was efficiently enhanced after the ECE. The rapid regrowth of *D. glomerata* during the recovery period might take advantage of the pool of accumulated WSCs in leaf meristematic tissues together with a putative enhancement of 6-FEH expression allowing the release of fructose through fructan breakdown. In addition, the strong recovery of *D. glomerata* could also be explained by the capacity of roots to take up nitrogen, as shown at the community level in previous studies (Roy *et al.*, 2016; Carlsson *et al.*, 2017) and reflected in our study by the high leaf nitrogen content in this species (Supplementary Fig. S1B). Moreover, this study shows the effects of eCO$_2$ and extreme stress over an entire growing season, but the long-term impact of the stimulated resilience identified for one species and for the entire plant community (Roy *et al.*, 2016) should be tested on a longer-term time scale due to a possible lag effect of ECE on biomass and species composition (Lee *et al.*, 2011; Zwicke *et al.*, 2013).

Although the ability of species to survive drastic climate change was claimed to be generally greater than hitherto recognized (Hof *et al.*, 2012), our results also highlight a strong inter-specific variability that should be further explored experimentally and taken into account in models. *D. glomerata*, although present at low abundance, responded in the same way as the whole plant community in the samples, whereas *H. lanatus* tended to disappear after the extreme climatic stress, hence modifying the overall species abundance, with potential long-term impacts on grassland properties linked to ecosystem services. Our results, showing strong effects of plant carbon, nitrogen, and water status, cast a light on the crucial and timely question of the mechanisms underlying plant resilience under extreme stress (Smith, 2011b; Nimmo *et al.*, 2015).

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Correlations between plant traits and resilience.

Fig. S1. Leaf nitrogen content in two grasses during and after an extreme climatic event.

Fig. S2. Monosaccharide contents in grass meristems during an extreme climatic event.

Fig. S3. Relative abundance of fructans in grass meristems during an extreme climatic event.

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