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1 **End-of-season senescence in grassland species can be traced to leaf**
2 **temperature during preceding summer drought**

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9 **Declaration of interest**

10 Conflicts of interest: none

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13 **Abstract**

14 In natural multi-species communities, drought extremes elicit complex, though seldom
15 measured, ecophysiological responses triggered by divergent drought coping strategies
16 and plant-plant interactions. This raises the question whether the whole-season impact
17 of such events is in any way predictable in such systems from stress measurements
18 during the drought. Here, we experimentally induce local variation in soil moisture in a
19 humid, multi-species, temperate grassland in summer and test whether any of the
20 stress indicators (stomatal conductance, net photosynthetic rate and midday leaf
21 surface temperature) measured on the seven most abundant species is a good early
22 warning signal of end-of-season senescence. We found that, across species, plants
23 exposed to lower soil water content experienced similar elevation of leaf surface
24 temperature, and that plants with warmer leaves during the drought extreme were
25 consistently more senescent at the end of the growing season two months later,
26 averaging 0.7% surplus leaf senescence with every additional 1°C. We also observed
27 links between lagged effects in the weeks after the drought, which were weakly negative
28 on stomatal conductance, but strongly positive on photosynthesis in some species, and
29 end-of-season senescence. Part of the damage might thus be ascribed to these drought
30 legacies. To conclude, even in complex field settings, local leaf surface temperature
31 measured at an early stage can be a powerful and species-specific indicator of the
32 whole-season impact of drought extremes. This opens perspectives to estimate where
33 in the landscape such events will be most detrimental.

34 **Keywords**

35 Individual-based measurements; Legacy effects; Field experiment; Stomatal
36 conductance; Leaf gas exchange; Photosynthesis.

38 **1. Introduction**

39 In multi-species communities, drought extremes elicit complex responses triggered by
40 divergent plant reactions and plant-plant interactions. As a consequence, the impact of
41 drought extremes in natural and semi-natural vegetation has mostly been investigated
42 on the ecosystem level, where lower-level processes are integrated. For example, many
43 studies have focused on whole-ecosystem biomass production and stability, in
44 response to either chronic (Isbell *et al.*, 2015) or pulsed (Pfisterer and Schmid, 2002;
45 Kahmen *et al.*, 2005; Vogel *et al.*, 2012) drought events. Ecophysiological research on
46 individual species remains underrepresented in these complex settings (McDowell,
47 2011) and is mostly limited to single crops (Maes and Steppe, 2012) where the canopy
48 surface is nowadays often remotely sensed for temperature (Swain *et al.*, 2012; Durigon
49 and de Jong van Lier, 2013) or spectral reflectance (Cebouni *et al.*, 1994; Haboudane
50 *et al.*, 2004) with the goal to determine degree of senescence, leaf area index,
51 chlorophyll concentration or general drought status. In multi-species field environments,
52 on the other hand, individual-based, species-specific ecophysiological indicators of
53 drought stress such as stomatal conductance, leaf water potential, photosynthetic
54 capacity, leaf surface temperature and chlorophyll fluorescence are thought to be
55 difficult to interpret in the field, given the multitude of possible interspecific interactions
56 (Allen and Allen, 1986; Craine and Dybzinski, 2013; Van den Berge *et al.*, 2014) and the
57 high susceptibility of some of these traits to microclimatic differences, variation in
58 weather conditions and plant morphology (Collatz *et al.*, 1991; Vogel, 2009).
59 Consequently, few studies have assessed such traits in multi-species field communities

60 in response to drought (Funk *et al.*, 2015; Guo *et al.*, 2017), let alone used them to
61 predict the resulting stress damage at an early stage.

62 During extreme drought, soil water potential drops to levels that excite stress responses
63 in plants, directed at survival. However, fundamental understanding of plant senescence
64 and death during drought extremes remains speculative (Bartlett *et al.*, 2016; Martin-
65 StPaul *et al.*, 2017; McDowell *et al.*, 2011). Former research has identified two main
66 causes. First, carbon starvation, resulting from photosynthetic or respiratory failure,
67 depletion of carbohydrate storage or limitations on phloem transport and carbohydrate
68 metabolism, and second, hydraulic failure due to embolism (Sala *et al.*, 2010). As such,
69 two main drought coping strategies have been observed, isohydry and anisohydry
70 (Sade *et al.*, 2012). These involve differences in stomatal behavior (Milbau *et al.*, 2005;
71 Daszkowska-Golec and Szarejko, 2013), down-regulation of photosynthesis (Jaleel *et*
72 *al.*, 2009; Reddy *et al.*, 2011), stimulation of root growth (Pilon *et al.*, 2013; Zwicke *et*
73 *al.*, 2015) and synthesis of protective molecules (Reddy *et al.*, 2004; Zandalinas *et al.*,
74 2018). Broadly speaking, isohydric species tend to close stomata quickly to avoid a drop
75 in leaf water potential and cavitation stress, at the cost of reducing carbon intake.
76 Anisohydric species are more drought stress tolerant and will keep stomata open longer
77 (Jones, 2007), thereby risking embolism but reducing the risk of carbon starvation. By
78 maintaining transpiration, anisohydric species also mitigate leaf surface heating, which
79 can exacerbate physiological stress during drought extremes (De Boeck *et al.*, 2011,
80 2016; Gielen *et al.*, 2007). The survival strategy used can vary between functional
81 groups (Elst *et al.*, 2016), between species (Van Peer *et al.*, 2004) and even within the
82 same species, depending on environment, climate and stress intensity/duration, and

83 intermediates between isohydry and anisohydry are common (Chaves *et al.*, 2010;
84 McDowell *et al.*, 2008).

85 The existence of these different survival strategies in terms of stomatal closure, rooting
86 depth, etc. complicates the interpretation of physiological measurements in the field for
87 two reasons: (1) a plant's survival is not only determined by its own stomatal behavior
88 (Kursar *et al.*, 2009), but also by the stomatal behavior of neighboring plants through
89 competition for water (Van den Berge *et al.*, 2014) and (2) variation in rooting depth in
90 mixed stands leads to complementarity and enhanced water uptake (Ashton *et al.*,
91 2010; Cardinale *et al.*, 2007; Dukes, 2001). During drought, this does not necessarily
92 enhance survival (Hernandez and Picon-Cochard, 2016; Silvertown *et al.*, 2014), as
93 plant-available water is reduced at a faster rate, increasing plant stress levels more
94 compared to single species stands as water deficits progress (Machado and Paulsen,
95 2001; Van Peer *et al.*, 2004; Verheyen *et al.*, 2008). Given these multiple factors of
96 complexity, it is challenging to assess the usefulness of physiological drought
97 responses in the field to understand the severity of drought and its resulting damage in
98 diverse communities. Therefore, is collecting data at the species level in such systems
99 really worth the effort?

100 In this study we experimentally induced local variation in soil moisture in a humid, multi-
101 species, temperate grassland community in the field, and measured drought-stress
102 responses (stomatal conductance, net photosynthetic rate and midday leaf surface
103 temperature during drought, as well as end-of season senescence) of the seven most
104 abundant species. We test whether any of the stress parameters taken during drought
105 can forecast the level of end-of-season senescence across species, and was thus a

106 good indicator of drought status at an early stage. Stomatal conductance and
107 photosynthesis are hypothesized not to be good predictive traits since stomatal closure
108 can both be advantageous or detrimental depending on the survival strategy used, as
109 outlined above, while leaf temperature is expected to have more predictive power as
110 elevated leaf temperatures can directly cause (heat) stress (Körner, 2003).

111

112 **2. Materials and Methods**

113 **2.1. Location and set-up**

114 In the summer of 2014, we created a gradient of six different soil moisture conditions in
115 a grassland in Halle, Belgium (51.249143 °N, 4.671710 °E, 11.2 m elevation). The
116 grassland is moist oligotrophic (N = 14.2 mg/kg; P = 194.0 mg/kg), has a sandy loam
117 soil (pF 0 = 52 ± 3 v%; pF 2 = 32 ± 5 v%; pF 4.2 = 4 ± 1 v%) and has been extensively
118 mown for more than 40 years. The seven most abundant species were selected as
119 study species and include common generalists (*Anthoxanthum odoratum* L., *Rumex*
120 *acetosa* L.), moist-adapted species (*Holcus lanatus* L., *Cardamine pratensis* L.,
121 *Ranunculus acris* L., *Ranunculus repens* L.) and wet-adapted (*Galium palustre* L.)
122 species (Ellenberg *et al.*, 1992).

123 We selected six 6 m x 8 m plots, and imposed an artificial drought by covering three of
124 them with a rain-out shelter. However, because we perceived a large naturally occurring
125 variation and range in volumetric soil water content (SWC) across the different plots
126 during drought, we chose to treat the six soil moisture conditions as a gradient. Shelters
127 consisted of a metal frame and a 0.18 mm thin polyethylene cover, attached at 0.8 m

128 above the vegetation to allow for air circulation and prevent heating. Previous research
129 demonstrated that the small microclimatic differences created by this type of shelter do
130 not lead to artifacts on plant responses in drought experiments (Kreyling *et al.*, 2016).
131 The cover excluded rainfall on a 4.4 m by 6.4 m patch within the plot, taking into
132 account maximum incoming rainfall angles of 45°. Around the shelters plastic was
133 inserted up to 20 cm deep to prevent water from seeping in. We assume that seepage
134 below 20 cm was low as (i) rainfall during the experiment (78 mm between 15 July and
135 8 September) was below average, (ii) August is the warmest month in the region, when
136 soils typically dry out, and (iii) we sampled on the aforementioned 4.4 m x 6.4 m plot
137 within the 6 m x 8 m shelter. The shelters were installed on July, 15th (DOY 197), the
138 middle of the local growing season (defined as the months with mean temperature
139 ($T_{\text{mean}} > 5^{\circ}\text{C}$ and precipitation [mm] $> 2 \times T_{\text{mean}} [^{\circ}\text{C}]$) to maximize drought impact. The
140 length of the rainfall exclusion period was initially chosen as the statistical 1000-year
141 recurrence of consecutive days with < 2.5 mm precipitation (59 days). However, an
142 extreme rain event of 41 mm on September 8th and 9th (DOY 251-252) followed by a
143 field-flooding largely leveled differences in soil moisture between the plots at 53 days
144 (see Results).

145 **2.2. Measurements**

146 Measurements were conducted at three different times during the experiment: one
147 month into the drought period from 20-25 August (DOY 232-237, period 1), one week
148 after the flood effectively ended the drought from 13-18 September (DOY 255-230,
149 period 2), and at the end of the growing season from 31 October – 1 November (DOY
150 305-306, period 3), seven weeks after the drought treatment had ended. SWC was

151 determined during period 1, 2 and 3 using PR2 Soil Moisture Profile Probes (Delta-T
152 Devices, Cambridge, UK), measuring at 10, 20 and 30 cm depth on 3 locations along
153 the lengthwise center line of the plot. During period 1 and 2 we measured stomatal
154 conductance (g_s) and light-saturated photosynthetic exchange rate (A_{sat}) with a portable
155 gas exchange system (LI-6400, LI-COR, Inc. Lincoln, NE, USA), using the 6400-02B
156 chamber for broad leaves, on the youngest fully expanded sun-exposed leaf of three
157 randomly chosen plants from each of the seven study species, in each of the six
158 different plots at $350 \mu\text{mol s}^{-1}$ air flow, 400 ppm CO_2 , ambient air temperature and
159 relative humidity, and saturating photosynthetically active radiation $1500 \mu\text{mol photons}$
160 $\text{m}^{-2} \text{s}^{-1}$. The g_s and A_{sat} values were logged after 2-5 min which was sufficient for
161 stabilization. All study species were C3-plants (Pyankov *et al.*, 2010), which are typically
162 light-saturated at around $600 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Nobel, 2009). There was no risk of
163 light-induced stress, since no shade species were present (Ellenberg *et al.*, 1992) and
164 all measurements were performed on sun-exposed leaves. We started measurements 3
165 h before solar noon, and continued until 3 h after solar noon (10:45 - 16:45). If the leaf
166 (or leaves in the case of *G. palustre*) did not cover the leaf chamber entirely, the leaf
167 edge was manually delineated on paper and the drawing's area digitally calculated
168 (Gimp 2.6.10, GNU) to correct g_s and A_{sat} . Also during period 1 and 2, leaf surface
169 temperature (T_{leaf}) was measured with a non-contact thermometer (TherMonitor C-1600,
170 LinearLaboratories, Fremont, USA) from 1 h before solar noon until 1 h after solar noon
171 (12:45 -14:45) on moments with direct solar radiation, likewise on one youngest fully
172 expanded leaf of three randomly chosen plants per species, in each of the six plots,
173 resulting in three T_{leaf} measurements per species per plot. We aimed the thermometer

174 perpendicular to the leaf surface at fully sun-exposed leaves, taking the reading from
175 the north to avoid shading by the instrument. In period 3, we visually estimated the
176 proportion of senescent leaf surface area (S) by assigning to it one of nine levels of
177 senescence (1%, 5%, 10%, 20%, 40%, 60%, 80%, 90% or 100% senescence, where
178 1% is quantified by few single dots of yellow or brown on an otherwise green leaf and
179 100% is a completely yellow or brown leaf). Senescence estimates were performed on
180 one randomly selected leaf of between 10 and 20 (depending on abundance within the
181 plot) randomly selected plants per species per plot (Cao *et al.*, 2015; Doley, 2010; Elst
182 *et al.*, 2016; Lemmens *et al.*, 2009; Poirier *et al.*, 2012). Every measurement was taken
183 on a different plant to avoid pseudoreplication and all measurements were rotated
184 between plots and species to neutralize the influence of changing weather conditions.

185 **2.3. Analysis**

186 To test for the effect of SWC on the various parameters, g_s , A_{sat} , T_{leaf} and S were
187 averaged per species per plot within each measurement period (given that SWC
188 measurements were not plant-specific). SWC was averaged per plot for each depth. To
189 determine whether we should use separate GLMs rather than MANOVA for all analyses
190 using g_s , A_{sat} and T_{leaf} as dependent variables, we determined the multicollinearity
191 among these physiological parameters. To test for multicollinearity between g_s , T_{leaf} and
192 A_{sat} in period 1 and 2, we performed MANOVA. Each column of the MANOVA's M
193 matrices used one separate dependent variable as transformation variable. We
194 performed univariate post-hoc F-tests on each dependent variable separately with a
195 MANOVA $P < 0.05$. On the basis of these tests, we decided to use separate GLMs for
196 subsequent analyses, since multicollinearity was high (Table A in appendix, Table 3).

197 For period 1, we tested the effect of SWC, species, SWC x depth, SWC x species and
198 SWC x depth x species on g_s , A_{sat} , and T_{leaf} with GLM. Effects of the same factors in
199 period 1 were tested on (end-of-season) S. Effects of SWC were analyzed at each
200 depth separately if SWC x depth showed $P < 0.05$, taking into account a Bonferroni-
201 corrected $\alpha = 0.017$. To identify lag effects on physiological parameters during recovery,
202 we also performed GLMs with SWC, species, SWC x depth, SWC x species and SWC x
203 depth x species of period 1 as independent variables and g_s , A_{sat} , and T_{leaf} of period 2
204 as dependent variables.

205 To investigate which physiological measurement would best account for the degree of
206 senescence at the end of the growing season, we tested the effects of A_{sat} , g_s and T_{leaf}
207 of periods 1 and 2 on end-of-season S with GLMM. Here, species was a random factor
208 since drought responses were not species-specific in previous analyses (no interaction
209 between species and SWC on the different parameters, Table 1).

210 All models were checked for normality of residuals with a Shapiro-Wilk test and
211 transformed if $W < 95$ (see Results for type of transformation). All statistics were
212 performed with JMP Pro 12.0.1. Except for the aforementioned analysis per depth, $\alpha =$
213 0.05.

215 **3. Results**

216 **3.1. Soil moisture gradient**

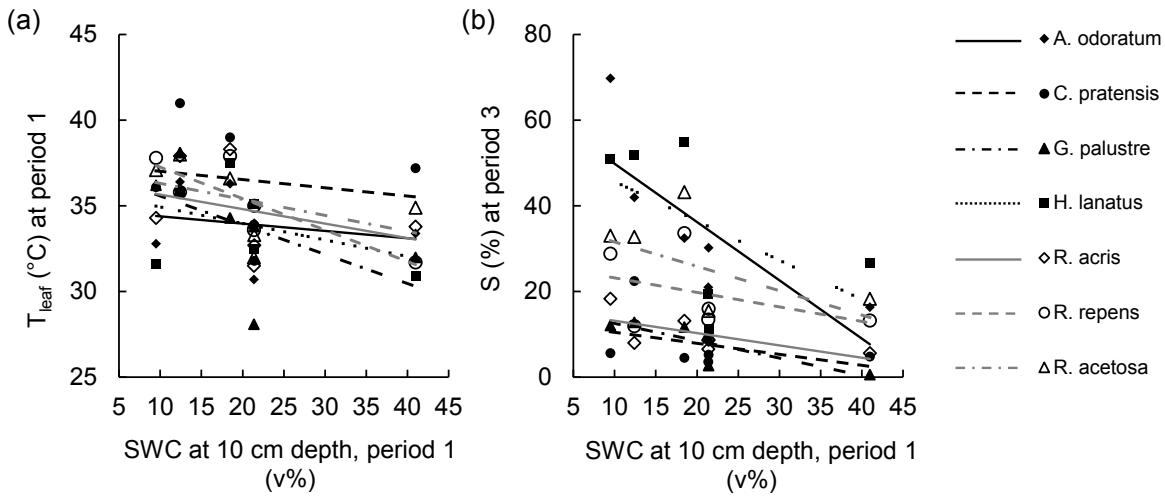
217 Large differences in SWC between the plots were apparent one month after the onset of
218 drought (DOY 232, period 1). At that time SWC measured at 10, 20 and 30 cm depth
219 ranged from 9.5 v% – 40.1 v%, 22.1 v% – 36.2 v% and 18 v% – 26.3 v%, respectively
220 (Fig. 1), showing that severe drought mostly developed in the top layer (wilting point pF
221 $4.2 = 4 \pm 1$ v%). A nearly complete lack of rainfall (11 mm) in subsequent days
222 prolonged drought up to an extreme rain event of 41 mm on DOY 251-252 (period 2).
223 One week after this event, SWC had shifted back towards field capacity (pF 2 = 32 ± 5
224 v%) at 10 cm depth, ranging from 13.4 v% – 40.1 v%, and approached or exceeded
225 field capacity at deeper levels, ranging from 23.3 v% – 38.3 v%. At the end of the
226 growing season, seven weeks after the treatment had ended (DOY 305, period 3),
227 readings largely exceeded field capacity, with plot averages per depth ranging from 36.4
228 v% – 53.3 v%.

229 **3.2. Drought effects**

230 At both periods 1 and 2, plants with lower g_s exhibited reduced photosynthesis and
231 warmer leaves across species (Table A in appendix, data not shown), pointing to high
232 multicollinearity between dependent variables of subsequent tests.

233 One month into the drought period, T_{leaf} varied widely from 28.1 – 41.0 °C (Fig. 1a).
234 Regardless of species identity, plants in plots with a lower SWC had higher T_{leaf} at that
235 time, and were more senescent at the end of the growing season (Fig. 1, Table 1).
236 These effects exclusively depended on SWC differences at 10 cm depth. Furthermore,

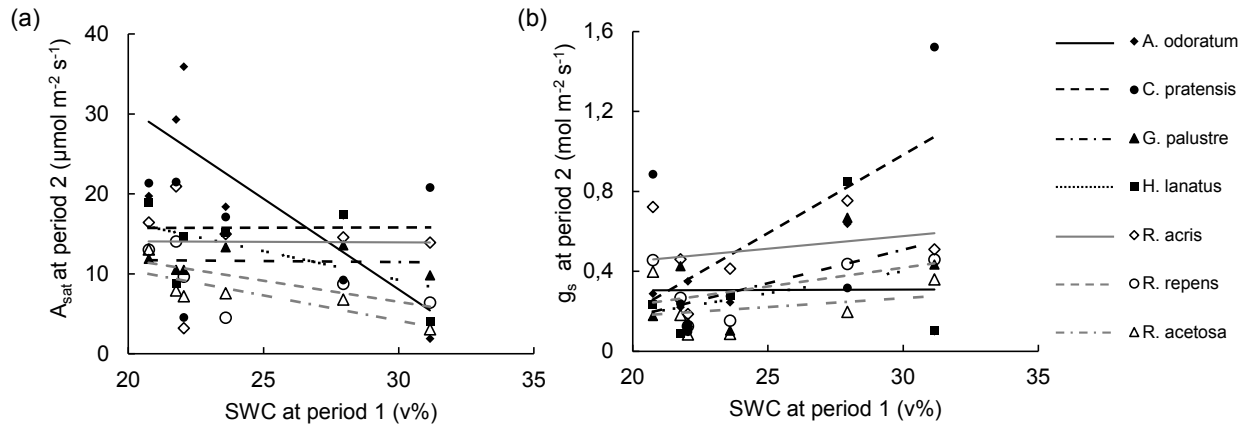
237 all plant stress measurements during drought, as well as end-of-season senescence,
 238 differed in magnitude between species as expected, but the responses to the
 239 differences in SWC did not (non-significant interaction SWC x species; Table 1).



240
 241 Fig. 1 (a) Leaf surface temperature (T_{leaf}) measured during drought (DOY 232-237, period 1) on fully expanded, sun-
 242 exposed leaves, on moments with direct solar radiation 1 h before to 1 h after solar noon and (b) senescence (S)
 243 expressed as the percentage of ungreen leaf surface, at the end of the growing season (DOY 305-306; period 3),
 244 both averaged per plot per species, and plotted against volumetric soil water content (SWC) at 10 cm depth during
 245 drought (DOY 232-237, period 1), averaged per plot. Separate regressions per species. Each symbol represents one
 246 species in one plot. R^2 and regression equations see Table B in appendix.

247 One week after the treatment, SWC no longer directly affected any of the response
 248 variables (Table 1, SWC and responses both measured in period 2), confirming that soil
 249 water was indeed sufficiently replenished. A_{sat} and g_s varied among species as
 250 expected, while the T_{leaf} readings had converged. However, plants in more severely
 251 droughted plots during the treatment (period 1) displayed lagged drought effects in the
 252 form of marginally significant ($P= 0.057$) lower g_s after drought (period 2) (Table 2, Fig.
 253 2). Photosynthetic activity, on the other hand, showed a significant, positive lagged

254 response to drought. Unlike all previous responses, this was species-specific (Table 2),
255 ranging from strong in some species to almost neutral in others (Fig. 2).

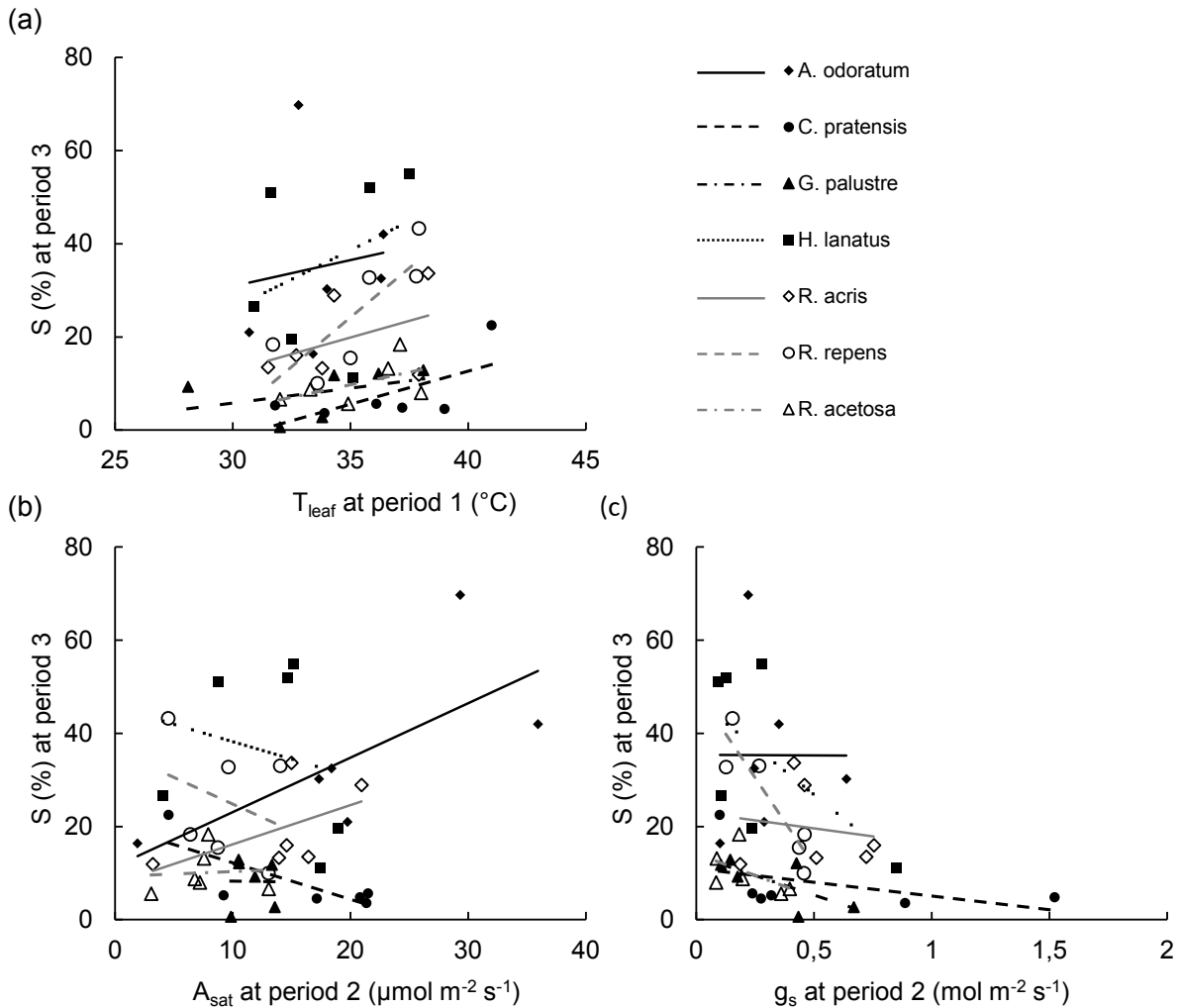


256

257 Fig. 2 Lagged drought effects. (a) Photosynthetic exchange (A_{sat}) and (b) stomatal conductance (g_s) one week after
258 the drought treatment (DOY 255-230, period 2), both measured 3 h before to 3 h after solar noon on fully expanded,
259 sun-exposed leaves at 400 ppm CO_2 , ambient air temperature and relative humidity, and saturating
260 photosynthetically active radiation $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, averaged per plot per species, regressed on volumetric
261 soil water content (SWC) during drought (DOY 232-237, period 1), averaged per plot. Separate regressions per
262 species. Each symbol represents one species in one plot. R^2 and equations see Table B in appendix.

263 3.3. Predictive value of physiological parameters

264 Plants with warmer leaves during the drought extreme (period 1) were consistently more
265 senescent at the end of the growing season (Table 3, Fig. 3a), while T_{leaf} measurements
266 taken one week after the drought (period 2) were no longer related to S (Table 3). In line
267 with previously stated lag effects though, a lower g_s measured one week after drought
268 did constitute a higher degree of S, while the A_{sat} level at that time had significant but
269 contrasting effects on S depending on species (Table 3, Fig. 3b, 3c).



270
 271 Fig. 3 Possible predictors of end-of-season senescence (S). S expressed as the percentage of ungreen leaf surface
 272 at the end of the growing season (DOY 305-306; period 3) plotted against (a) leaf surface temperature (T_{leaf})
 273 measured on fully expanded, sun-exposed leaves, on moments with direct solar radiation 1 h before to 1 h after solar
 274 noon during drought (DOY 232-237, period 1), and plotted against (b) photosynthetic exchange (A_{sat}) and (c) stomatal
 275 conductance (g_s) one week after the drought treatment (DOY 255-230, period 2), both measured 3 h before to 3 h
 276 after solar noon on fully expanded, sun-exposed leaves at 400 ppm CO_2 , ambient air temperature and relative
 277 humidity, and saturating photosynthetically active radiation 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, averaged per plot per species.
 278 Separate regressions per species; each symbol represents one species in one plot. R^2 and equations see Table B in
 279 appendix.

281 **4. Discussion**

282 **4.1. End-of-season senescence is signaled by leaf surface temperature**

283 Across species, elevated leaf temperature during the drought extreme was a powerful
284 and early predictor of higher senescence by the end of the season two months later.
285 Every 1°C of additional heat induced 0.7% surplus damage, regardless of species. This
286 finding is remarkable, and compelling for several reasons: (1) The correlation holds
287 across species differing in both functional group (grasses and herbs) and soil moisture
288 preference (Ellenberg, 1992, see Materials and Methods). In addition, the impact of
289 drought, and consequentially leaf warming, on end-of-season leaf senescence is highly
290 species-dependent since it hinges on species-specific traits such as the onset and
291 progression of phenological senescence (Rivero *et al.*, 2007; Sade *et al.*, 2018; Wu *et*
292 *al.*, 2012) and the production of heat shock proteins (Halter *et al.*, 2017). (2) The effect
293 subsists under field conditions which are subject to weather variation (Vogel, 2009),
294 microclimatic differences on a small scale (Collatz *et al.*, 1991) and multiple abiotic
295 stressors (Chaves *et al.*, 2002), as well as to positive and negative biotic interactions
296 (Ramegowda and Senthil-Kumar, 2015). Moreover, any microclimatic differences
297 caused by the rain-out shelters did not preclude the detection of this pattern, in line with
298 aforementioned study by Kreyling *et al.* (2016). (3) Leaf temperature was measured at a
299 time when SWC in the driest plots approached wilting point conditions only up to 10 cm
300 depth. While species may root relatively superficially in this moist grassland (Schenk &
301 Jackson, 2002), it is unlikely that all species perceived a large degree of stress at this
302 time.

303 **4.2. Uniform leaf heating in response to drought**

304 At the basis of our key finding, we observed several interesting ecophysiological
305 patterns. Plants exposed to a lower SWC experienced similar leaf heating regardless of
306 species identity (Boulet *et al.*, 2007; Goward *et al.*, 2002; Li *et al.*, 2008), in contrast with
307 the wide variation in drought coping strategy generally observed in multi-species
308 communities (Elst *et al.*, 2016; Milbau *et al.*, 2005; Zwicke *et al.*, 2015). Yet our
309 grassland was moist, and such grasslands typically harbor species characterized by
310 isohydric responses (McDowell *et al.*, 2008), which would explain the observed
311 uniformity of the temperature increase. Isohydric responses avoid rapid aggravation of
312 soil water shortage by excessive transpiration but in the process prevent leaf
313 evaporative cooling, so stress impact would be dominated relatively more by heat
314 compared with anisohydric species. Unfortunately we could not precisely pinpoint the
315 dominant stomatal response (isohydric or anisohydric) due to large variability in the g_s
316 readings, which may originate from genetic variation within species (Geber and
317 Dawson, 1997), soil heterogeneity (Martorell *et al.*, 2015), microclimatic variations
318 (Collatz *et al.*, 1991), effects of neighbor identity (Van den Berge *et al.*, 2014) and even
319 the restricted number of replicates, illustrating that ecophysiological measurements in
320 diverse natural communities are not without limitations, and should be accompanied by
321 microclimatic observations in future approaches. However, there are alternative
322 explanations for the observed convergence in leaf heating: mixing of the air and
323 associated heat transfer between plants, or heating of the soil and subsequent heat
324 transfer to the plants may have exposed all individuals within a plot to roughly the same
325 warming (Jones and Rotenberg, 2011).

326 As mentioned above, the relationship between leaf temperature during the drought and
327 leaf mortality seven weeks after the drought had ended was surprisingly uniform across
328 species. However, this matches the remarkably similar temperature threshold for heat
329 tolerance of around 40 °C across plant species even from different biomes (Körner,
330 2003). Indeed, one month after the start of the treatment, we already observed leaf
331 temperatures up to 41 °C, and an even further divergence in drought status and canopy
332 temperature between plots before drought termination by heavy rain cannot be
333 excluded. Furthermore, drought could have triggered a consistently earlier onset of
334 phenological senescence across species at plots subjected to drier conditions (Bloor *et*
335 *al.*, 2010; Jentsch *et al.*, 2009; Morales *et al.*, 2013). Though this process is species-
336 dependent, the impact of drought on phenological senescence would, in this instance,
337 have outweighed any among-species variation.

338 **4.3. The role of lagged physiological effects in end-of-season senescence**

339 One week after the end of the drought period, a weak legacy effect of reduced stomatal
340 conductance remained. This minor effect may nevertheless be important because end-
341 of-season senescence was clearly inversely related to g_s at this time. Moreover, several
342 studies report that recovery rates of g_s are inversely related to drought intensity
343 (Blackman *et al.*, 2009; Liang *et al.*, 2002; Miyashita *et al.*, 2005; Xu *et al.*, 2010), so
344 weak initial differences between plots might diverge so long as physiological recovery is
345 not completed. Part of the damage might therefore be ascribed to lagged effects of
346 drought (Van De Velde *et al.*, 2015). Legacy effects on photosynthesis, on the other
347 hand, were distinct but ranged from absent to strongly positive depending on species. A
348 higher photosynthetic rate in previously dry plots may seem counterintuitive, but has

349 been observed before after rewetting of dry soil (Dreesen *et al.*, 2012) and could be
350 ascribed to an increased mineralization of either dead microbial biomass or
351 osmoregulatory substances released by soil microorganisms in response to hypo-
352 osmotic stress in plots subjected to more severe drought, the so-called 'Birch-effect'
353 (Borken and Matzner, 2009; Jarvis *et al.*, 2007; Unger *et al.*, 2010). Measuring pre-
354 drought and pre- and post-rewetting soil nutrient availability would therefore be
355 interesting in future studies to unravel its potential role. The two mechanisms proposed
356 here are not mutually exclusive, and inverse responses of g_s and A_{sat} are possible, in
357 particular under nutrient-limiting rather than CO_2 -limiting conditions (Murray *et al.*, 2000;
358 Yamori *et al.*, 2011). Indeed, initial plant-available soil N content was low in this
359 oligotrophic grassland (see '2.1. Location and set-up').

360 Contrary to expectation, the effects of A_{sat} after rewetting on end-of-season senescence
361 were not always positive (Fig. 3b). While the lagged positive effect of drought on A_{sat}
362 (Fig. 2a) may have buffered the stress-induced senescence in some of the species, a
363 higher photosynthetic rate could not induce enough regrowth to mask the damage
364 resulting from (direct or lagged) drought stress. Indeed, the drought treatment started at
365 the peak of the growing season, limiting the timeframe in which post-drought regrowth
366 could occur (Denton *et al.*, 2016). Moreover, the negative effects of a higher
367 photosynthetic rate on end-of-season senescence in some species (Fig. 3b) suggest
368 that enhanced supply of carbohydrates does not always compensate for hydric stress
369 (Granda and Camarero, 2017).

370 In conclusion, we demonstrated that in semi-natural vegetation with all its associated
371 complexity, differences between landscape patches in the whole-season impact of an

372 extreme drought can be predicted from corresponding differences in plant temperature
373 between these patches, at a relatively early stage of the event when drought is still
374 superficial. Clearly, temperatures should be compared within species and other
375 ecosystems might see different degrees of soil water shortage and warming, yet the
376 uniform heat tolerance of plants reported in literature suggests that the coupling
377 between stress-induced senescence and high temperature as observed across species
378 in our experiment could be more broadly valid. We therefore suggest to conduct similar
379 experiments also in other ecosystems, ideally using a multi-annual approach, for
380 example with new plot locations representing a drought gradient each year.

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644

646 **7. Tables**

647 Table 1 P-values of GLM on the effects of species identity and soil water content (SWC) in period 1 and 2 on the
 648 respective period's light-saturated photosynthetic exchange rate (A_{sat}), stomatal conductance (g_s) and leaf surface
 649 temperature (T_{leaf}) as well as on end-of-season senescence (S). Significant values in bold. When interaction of SWC
 650 with depth was significant, separate GLM's for each depth are shown ($P < 0.017$). df are df_{model} , df_{error} . F-values see
 651 Table C in appendix.

Period 1	df	$\sqrt{A_{sat}}$	g_s	T_{leaf}	S (end of season)
SWC	1, 98	P = 0.570	P = 0.500	P = 0.014	P = 0.002
Species	6, 98	P < 0.001	P < 0.001	P = 0.019	P < 0.001
SWC x depth	2, 98	P = 0.962	P = 0.004	P = 0.007	P = 0.037
SWC at 10 cm depth	1, 28		P = 0.64	P = 0.012	P = 0.015
SWC at 20 cm depth	1, 28		P = 0.030	P = 0.164	P = 0.587
SWC at 30 cm depth	1, 28		P = 0.012	P = 0.035	P = 0.443
SWC x species	6, 98	P = 0.865	P = 0.419	P = 0.945	P = 0.647
SWC x depth x species	12, 98	P = 0.370	P = 0.569	P = 0.967	P = 0.965
Period 2	df	A_{sat}	Ln g_s	Ln T_{leaf}	S (end of season)
SWC	1, 98	P = 0.116	P = 0.819	P = 0.182	P = 0.657
Species	6, 98	P < 0.001	P = 0.012	P = 0.959	P < 0.001
SWC x depth	2, 98	P = 0.556	P = 0.114	P = 0.110	P = 0.191
SWC x species	6, 98	P = 0.679	P = 0.746	P = 0.993	P = 0.990
SWC x depth x species	12, 98	P = 0.040	P = 0.717	P = 1,000	P = 0.979

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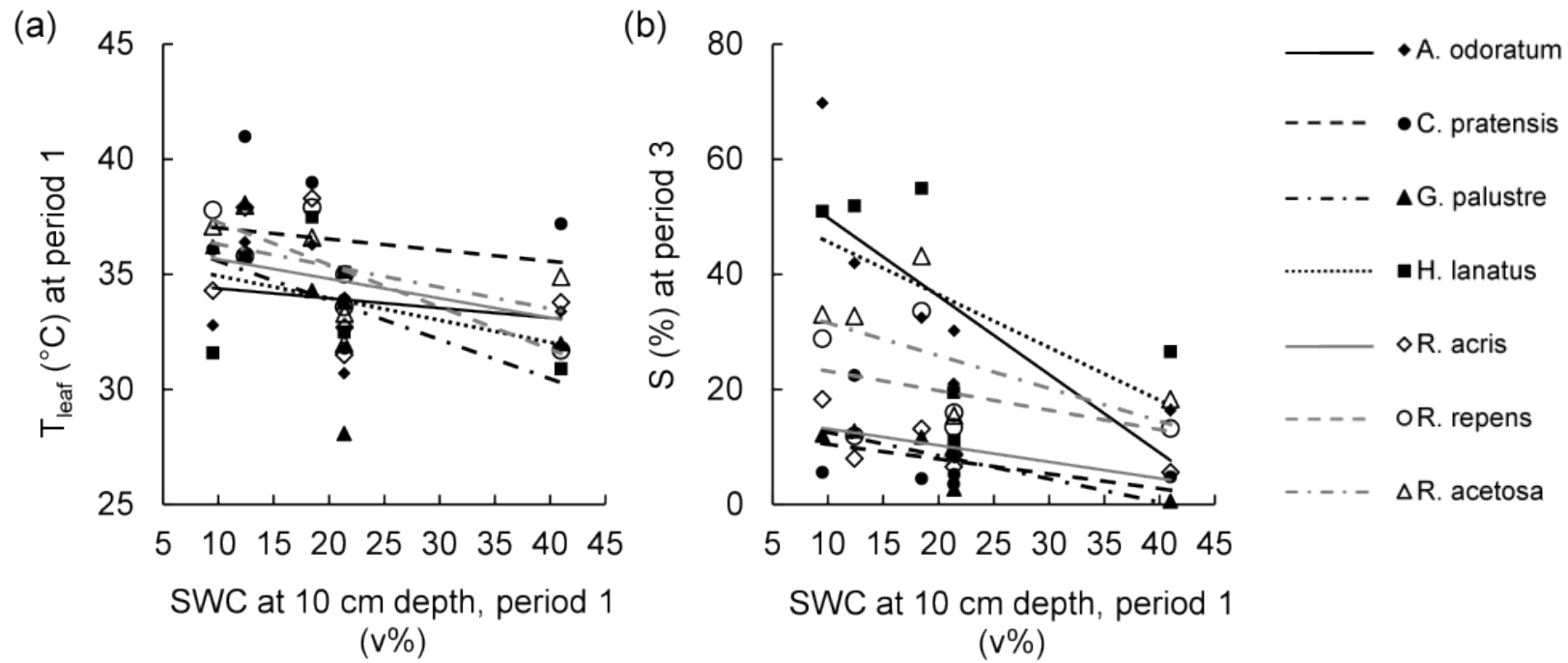
654 Table 2 Lagged drought effects. P-values of GLM on the effects of soil water content (SWC) and species identity
 655 during the drought (period 1) on photosynthetic exchange (A_{sat}), stomatal conductance (g_s) and leaf surface
 656 temperature (T_{leaf}) one week after drought (period 2). Significant values in bold (df are df_{model} , df_{error}). F-values see
 657 Table D in appendix.

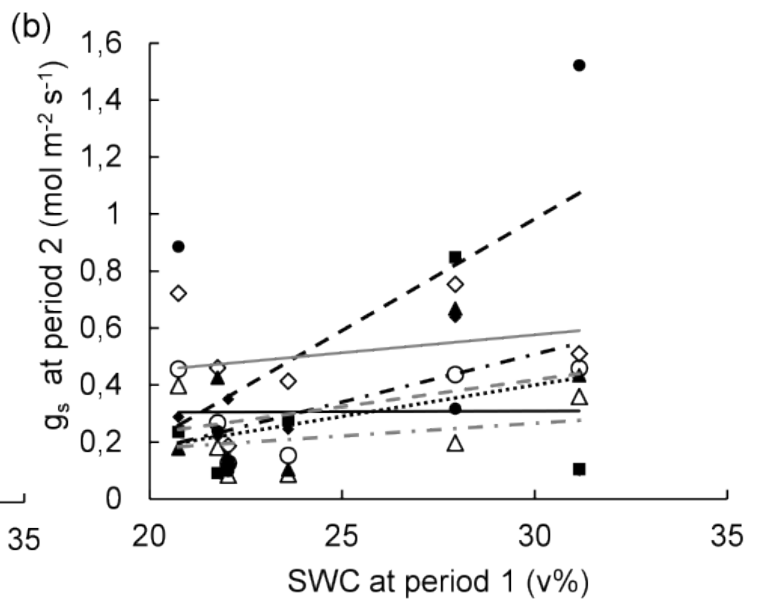
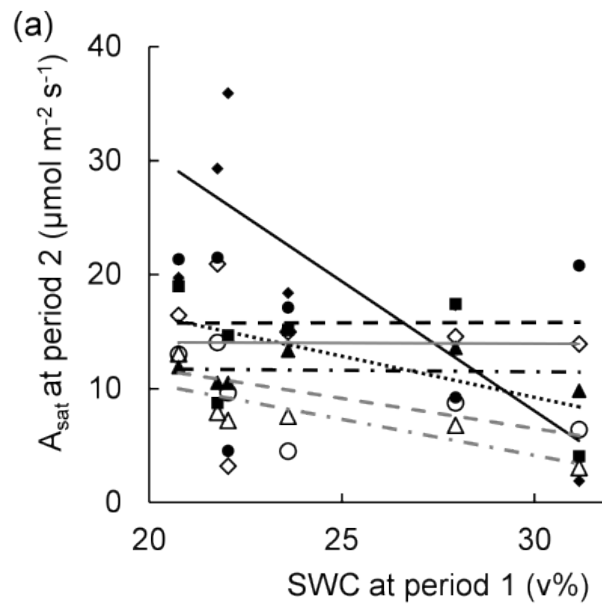
Lag effects	df	A_{sat}	Ln g_s	Ln T_{leaf}
SWC	1, 98	P = 0.042	P = 0.057	P = 0.065
Species	6, 98	P < 0.001	P < 0.001	P = 0.954
SWC x depth	2, 98	P = 0.297	P = 0.598	P = 0.083
SWC x species	6, 98	P = 0.026	P = 0.199	P = 9.999
SWC x depth x species	12, 98	P = 0.569	P = 0.357	P = 9.999

658
 659 Table 3 P-values of GLMM on the effects of photosynthetic exchange (A_{sat}), stomatal conductance (g_s) and leaf
 660 surface temperature (T_{leaf}) measured in period 1 and 2 on end-of-season senescence (S). Significant values in bold
 661 (df are df_{model} , df_{error}). F-values see Table E in appendix.

	Period 1		Period 2	
	df	Ln S	df	S
A_{sat}	1, 34	P = 0.242	1, 37	P = 0.009
g_s	1, 34	P = 0.730	1, 34	P = 0.004
T_{leaf}	1, 33	P = 0.040	1, 32	P = 0.349

662





- ◆— *A. odoratum*
- - -●- *C. pratensis*
- · - · -▲- *G. palustre*
- *H. lanatus*
- ◇— *R. acris*
- - -○- *R. repens*
- · - · -△- *R. acetosa*

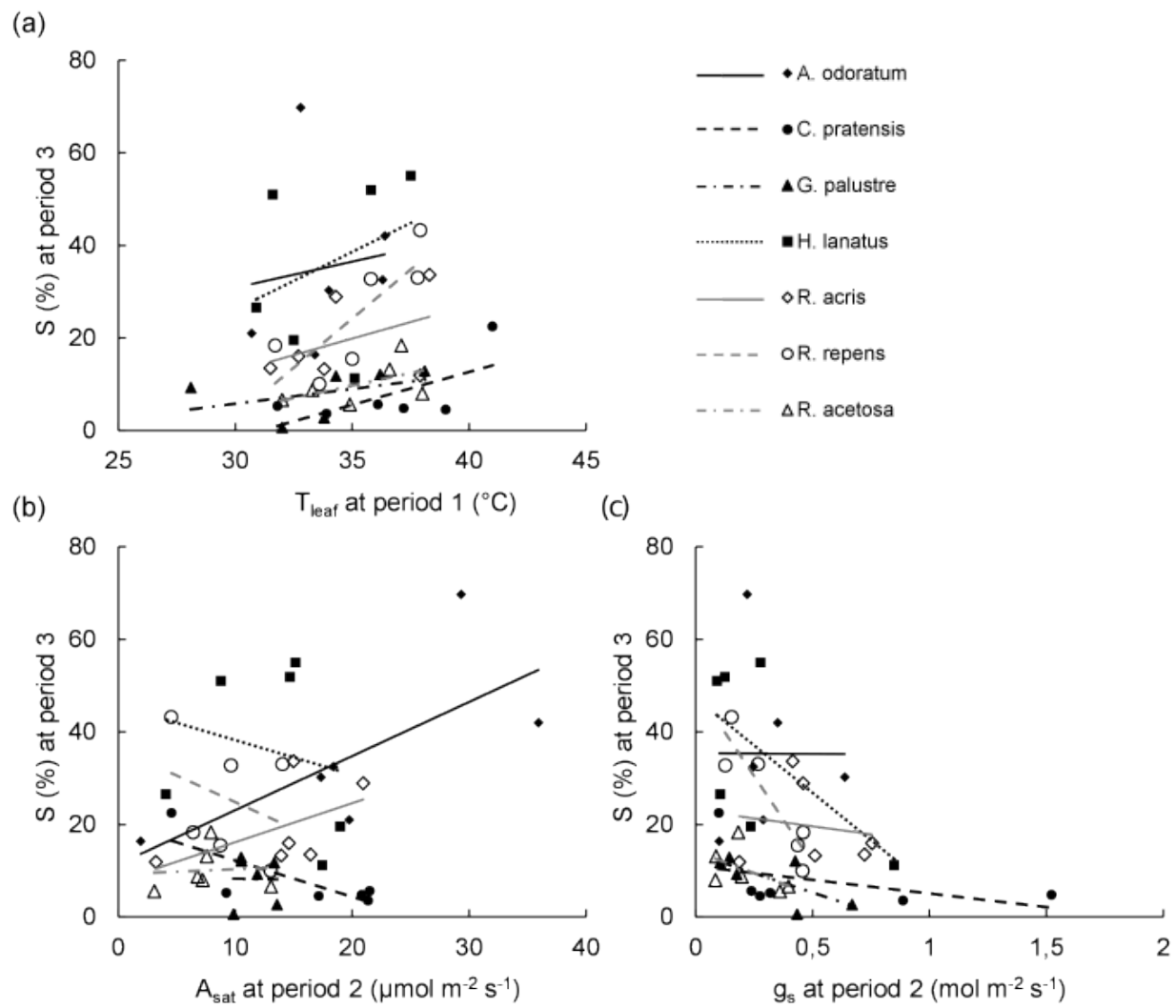


Fig. 1 (a) Leaf surface temperature (T_{leaf}) measured during drought (DOY 232-237, period 1) on fully expanded, sun-exposed leaves, on moments with direct solar radiation 1 h before to 1 h after solar noon and (b) senescence (S) expressed as the percentage of ungreen leaf surface, at the end of the growing season (DOY 305-306; period 3), both averaged per plot per species, and plotted against volumetric soil water content (SWC) at 10 cm depth during drought (DOY 232-237, period 1), averaged per plot. Separate regressions per species. Each symbol represents one species in one plot. R^2 and regression equations see Table B in appendix.

Fig. 2 Lagged drought effects. (a) Photosynthetic exchange (A_{sat}) and (b) stomatal conductance (g_s) one week after the drought treatment (DOY 255-230, period 2), both measured 3 h before to 3 h after solar noon on fully expanded, sun-exposed leaves at 400 ppm CO_2 , ambient air temperature and relative humidity, and saturating photosynthetically active radiation $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, averaged per plot per species, regressed on volumetric soil water content (SWC) during drought (DOY 232-237, period 1), averaged per plot. Separate regressions per species. Each symbol represents one species in one plot. R^2 and equations see Table B in appendix.

Fig. 3 Possible predictors of end-of-season senescence (S). S expressed as the percentage of ungreen leaf surface at the end of the growing season (DOY 305-306; period 3) plotted against (a) leaf surface temperature (T_{leaf}) measured on fully expanded, sun-exposed leaves, on moments with direct solar radiation 1 h before to 1 h after solar noon during drought (DOY 232-237, period 1), and plotted against (b) photosynthetic exchange (A_{sat}) and (c) stomatal conductance (g_s) one week after the drought treatment (DOY 255-230, period 2), both measured 3 h before to 3 h after solar noon on fully expanded, sun-exposed leaves at 400 ppm CO_2 , ambient air temperature and relative humidity, and saturating photosynthetically active radiation $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, averaged per plot per species. Separate regressions per species; each symbol represents one species in one plot. R^2 and equations see Table B in appendix.

Table A P- and F-values of MANOVA and subsequent univariate F-tests on the effect of stomatal conductance (g_s) on photosynthetic exchange (A_{sat}) and leaf surface temperature (T_{leaf}) in periods 1 and 2. Significant values in bold (**P < 0.01; ***P < 0.001; df are df_{model} , df_{error}).

	Multivariate		Post-hoc univariate		
	df	MANOVA	df	Ln A_{sat}	T_{leaf}
Period 1					
g_s	2, 123	P < 0.001 F = 36.09***	1, 124	P < 0.001 F = 41.21***	P < 0.001 F = 38.62***
Period 2					
g_s	2, 123	P < 0.001 F = 13.57***	1, 124	P < 0.001 F = 25.09***	P = 0.003 F = 9.18**

Table B R²-values and regression equations of Fig. 1, 2 and 3 per study species.

	Fig. 1a	Fig. 1b	Fig. 2a	Fig. 2b	Fig. 3a	Fig. 3b	Fig. 3c
<i>A. odoratum</i>	R ² = 0.05 y = -0.043x + 34.81	R ² = 0.62 y = -1.361x + 63.462	R ² = 0.64 y = -2.271x + 76.17	R ² < 0.01 y = 0.001x + 0.30	R ² = 0.02 y = 1.132x - 3.10	R ² = 0.51 y = 1.170x + 11.40	R ² < 0.01 y = -0.240x + 35.39
<i>C. pratensis</i>	R ² = 0.03 y = -0.048x + 37.49	R ² = 0.15 y = -0.255x + 13.00	R ² < 0.01 y = 0.008x + 15.57	R ² = 0.35 y = 0.078x - 1.37	R ² = 0.43 y = 1.421x - 44.14	R ² = 0.60 y = -0.784x + 20.08	R ² = 0.20 y = -5.909x + 11.01
<i>G. palustre</i>	R ² = 0.29 y = -0.170x + 37.27	R ² = 0.72 y = -0.405x + 16.60	R ² < 0.01 y = -0.023x + 12.18	R ² = 0.39 y = 0.034x - 0.50	R ² = 0.18 y = 0.646x - 13.59	R ² < 0.01 y = -0.026x + 8.52	R ² = 0.50 y = -16.799x + 13.68
<i>H. lanatus</i>	R ² = 0.17 y = -0.097x + 35.89	R ² = 0.72 y = -0.919x + 54.89	R ² = 0.28 y = -0.723x + 30.92	R ² = 0.10 y = 0.022x - 0.26	R ² = 0.12 y = 2.503x - 48.97	R ² = 0.05 y = -0.746x + 45.70	R ² = 0.39 y = -41.026x + 47.37
<i>R. acris</i>	R ² = 0.11 y = -0.085x + 36.51	R ² = 0.16 y = -0.338x + 26.53	R ² < 0.01 y = -0.011x + 14.29	R ² = 0.06 y = 0.013x + 0.20	R ² = 0.18 y = 1.427x - 30.03	R ² = 0.28 y = 0.843x + 7.73	R ² = 0.02 y = -6.819x + 23.01
<i>R. repens</i>	R ² = 0.71 y = -0.184x + 39.10	R ² = 0.24 y = -0.568x + 37.22	R ² = 0.34 y = -0.529x + 22.39	R ² = 0.25 y = 0.019x - 0.15	R ² = 0.64 y = 4.239x - 124.16	R ² = 0.11 y = -1.147x + 36.26	R ² = 0.84 y = -75.513x + 49.34
<i>R. acetosa</i>	R ² = 0.20 y = -0.094x + 37.25	R ² = 0.44 y = -0.288x + 16.03	R ² = 0.66 y = -0.631x + 23.09	R ² = 0.07 y = 0.01x + 0.00	R ² = 0.27 y = 1.069x - 27.68	R ² < 0.01 y = 0.110x + 9.24	R ² = 0.27 y = -18.586x + 14.13

Table C F-values of GLM on the effects of species identity and soil water content (SWC) in period 1 and 2 on the respective period's light-saturated photosynthetic exchange rate (A_{sat}), stomatal conductance (g_s) and leaf surface temperature (T_{leaf}) as well as on end-of-season senescence (S). Significant values in bold (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). When interaction of SWC with depth was significant, separate GLM's for each depth are shown (* $P < 0.017$). df are df_{model} , df_{error} .

Period 1	df	$\sqrt{A_{\text{sat}}}$	g_s	T_{leaf}	S (end of season)
SWC	1, 98	F = 0.32	F = 0.77	F = 6.22*	F = 10.00**
Species	6, 98	F = 9.04***	F = 8.75***	F = 2.66*	F = 13.75***
SWC x depth	2, 98	F = 0.04	F = 5.85**	F = 5.30**	F = 3.42*
SWC at 10 cm depth	1, 28		F = 0.22	F = 6.94*	F = 6.39*
SWC at 20 cm depth	1, 28		F = 5.05	F = 2.01	F = 0.30
SWC at 30 cm depth	1, 28		F = 6.88*	F = 4.75	F = 0.60
SWC x species	6, 98	F = 0.41	F = 1.02	F = 0.27	F = 0.70
SWC x depth x species	12, 98	F = 1.10	F = 0.88	F = 0.38	F = 0.39
Period 2	df	A_{sat}	Ln g_s	Ln T_{leaf}	S (end of season)
SWC	1, 98	F = 2.25	F = 0.05	F = 1.81	F = 0.19
Species	6, 98	F = 10.08***	F = 2.90*	F = 0.25	F = 15.30***
SWC x depth	2, 98	F = 0.59	F = 2.22	F = 2.26	F = 1.68
SWC x species	6, 98	F = 0.66	F = 0.57	F = 0.12	F = 0.14
SWC x depth x species	12, 98	F = 1.93*	F = 0.73	F = 0.04	F = 0.34

Table D Lagged drought effects. F-values of GLM on the effects of soil water content (SWC) and species identity during the drought period (period 1) on photosynthetic exchange (A_{sat}), stomatal conductance (g_s) and leaf surface temperature (T_{leaf}) one week after drought (period 2). Significant values in bold ((* $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; df are df_{model} , df_{error}).

Lag effects	df	A_{sat}	Ln g_s	Ln T_{leaf}
SWC	1, 98	F = 4.24*	F = 3.72(*)	F = 3.48(*)
Species	6, 98	F = 6.41***	F = 4.20***	F = 0.26
SWC x depth	2, 98	F = 1.23	F = 0.52	F = 2.56 (*)
SWC x species	6, 98	F = 2.52*	F = 1.46	F = 0.04
SWC x depth x species	12, 98	F = 0.88	F = 1.11	F = 0.07

Table E F-values of GLMM on the effects of photosynthetic exchange (A_{sat}), stomatal conductance (g_s) and leaf surface temperature (T_{leaf}) measured in period 1 and 2 on end-of-season senescence (S). Significant values in bold (* $P < 0.05$; ** $P < 0.01$; df are df_{model} , df_{error}).

	Period 1		Period 2	
	df	Ln S	df	S
A_{sat}	1, 34	F = 1.42	1, 37	F = 7.60**
g_s	1, 34	F = 0.12	1, 34	F = 9.32**
T_{leaf}	1, 33	F = 4.56*	1, 32	F = 0.90